

POTENTIALITIES OF NIRMALI SEEDS IN THE CLARIFICATION OF WATER AND WASTE WATER

A Thesis Submitted
In Partial Fulfilment of the Requirements
for the Degree of
MASTER OF TECHNOLOGY

By
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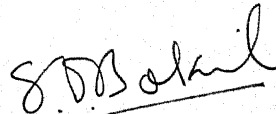
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CERTIFICATE

Certified that the work presented in this thesis entitled 'Potentialities of Nirmali Seeds in the Clarification of Water and Waste Water' by Mr. P.N. Tripathi has been carried out under my supervision and it has not been submitted elsewhere for a degree.



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ABSTRACT

Nirmali seed (strychnos potatorum) was found to be anionic polyelectrolyte having surface groups ($-\text{COOH}$) and ($-\text{OH}$) as determined by I.R. Spectral Studies. The main constituents of the seed are carbohydrate, protein and lipid. Inorganic content is very low only about 2 percent.

In a detailed study consisting of jar tests and plate counts on synthetic as well as natural turbid samples, Nirmali seed extract was found to be very efficient in the flocculation of hydrophobic colloids (such as clay turbidity) but a poor flocculant in the case of hydrophilic colloids like bacteria, algae and colloids in raw sewage (mostly organic in nature). The efficiency of removal of turbidity increases with the increase in raw water turbidity. The optimum pH was found to be nearly 7.5 and optimum dose was 2 mg/l for raw water turbidity of 500 FTU. The dose varies with the concentration of turbidity. Also efficiency of turbidity removal increases with the increase in Ca^{++} ion concentration.

Nirmali seed extract can also be used as coagulant aid. Saving in alum was 50 to 55 percent in the case of

inorganic turbidity, 30 to 35 percent in the case of algae, and no significant saving in a mixed suspension of bacteria and inorganic turbidity.

The test for surface tension measurement showed 20 percent decrease in surface tension of water after addition of Nirmali seed extract. This indicates that the removal of hydrophobic colloids from water by Nirmali seed extract may be by a mechanism analogous to that of detergents.

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CHAPTER I

INTRODUCTION

1.1 Introduction

The engineering management of coagulation by the use of synthetic and natural polyelectrolytes is the most recent development in the clarification of drinking waters. However, the use of natural polyelectrolytes was known to ancient Indians as far back as 4000 years. In Sanskrit literature (about 2000 B.C.), use of many plant substances, notably Nirmali seed (Stryconos potatorum), is mentioned as a means of clarifying water. Now, it is evident that such organic compounds as starch and starch derivatives, cellulose compounds, polysaccharide, gums, and proteinaceous materials may be used as coagulants or coagulant aids. In spite of the variations in the source of these compounds, they may be collectively described as biocolloids which are naturally occurring polymers carrying electric charges or ionizable groups. An important development in the field of colloidal science is the production of synthetic polymers with high molecular weights and whose general behaviour is analogous to the above mentioned biocolloids

All of these compound contain a number of recurring units of small molecular weight, chemically combined to form a molecule of colloid size, and each of the recurring units carries one or more electric charges or ionizable groups. As these compounds have the characteristics of both polymers and electrolytes they are frequently called as polyelectrolytes.

Use of polyelectrolytes in water treatment is gaining momentum. Recently Environmental protection agency (U.S.A.) has recommended the names of 134 synthetic polyelectrolytes that can be used as coagulant aids in water treatment (20). In India Central Public Health Engineering Research Institute (CPHERI) has also developed some natural and synthetic polyelectrolytes (5,6) keeping in view the shortage and growing cost of metal coagulant. Cost of alum being used at Kanpur Water Works has gone up from Rs. 280/Tonne last year to Rs. 650/ Tonne this year. But presently, most of the polyelectrolytes are rather costly and many have to be imported.

Nirmali seed which is an indigenous product is reported to be very efficient both as coagulant (8,9) and coagulant aid (7,10) in the removal of turbidity from natural waters. Surface waters generally contain both

inorganic as well as organic and biological turbidity, the latter being caused by a wide variety of micro-organisms usually present in normal surface waters. Though these organisms may not be actually pathogenic to man, the main complaints against them are that they interfere in the processes of filtration, disinfection etc., and that they are unacceptable on the grounds of palatability of water and its aesthetics. These organisms may accumulate in such numbers that water may become unshightly and turbid and many of the organisms do impart disagreeable odour and colour to water (11) Although detection of coliform bacteria is of primary importance, attention must also be directed towards controlling the general bacterial population. The chemicals used in the clarification process such as coagulation and flocculation must be considered on the basis of their merits. Various alternatives must be considered in order to develop sound, dependable treatment programs (13).

1.2 Objectives of the Present Study

In any removal process whether it is physical, chemical or biological, the most fundamental and important part of the process is to know the mechanism of removal.

But from the previous studies about turbidity removal by the Nirmali seed extract it appears that the mechanism is not yet well defined. There is uncertainty whether the polyelectrolyte is anionic cationic or non ionic. So the first objective of this study is the characterization of Nirmali seed extract.

Secondly, most of the natural water contain dirt as well as bacteria and aquatic plants like algae. Previous investigators did not care to think about their removal. Bacteria, algae, etc are more serious pollutants than the simple inorganic turbidity. The organisms that may cause, or have been known to cause problem in water supply, include several species of algae, protozoa, and diatom that produce taste and odors and clog filters, iron bacteria that produce tastes, and odors and clog pipes. Therefore, the second objective of this work is to study the effectiveness of nirmali seed extract in the removal of bacteria and algae from natural waters.

There are conflicting reports about the suitability of the Nirmali extract as the sole coagulant. The third objective of this study, therefore, is to explore the suitability of Nirmali seed as a coagulant or failing which to study it as a coagulant aid along with the usual standard coagulant, such as alum.

CHAPTER II

COAGULATION IN WATER AND WASTE WATER TREATMENT

2.1 Coagulation and Flocculation

Coagulation and flocculation have been interpreted differently in chemical and engineering literature. According to LaMer, coagulation refers to destabilization produced by compression of the electric double layers surrounding all colloidal particles, while flocculation refers to destabilization by the adsorption of large organic polymers and the subsequent formation of particle-polymer particle bridges. This distinction can have practical significance, since colloid aggregation involving destabilization by double layer compression occurs, at a constant concentration of coagulant, regardless of the concentration of colloidal material. In contrast, colloidal aggregation involving destabilization by the adsorption of large polymers can involve a linear (stoichiometric) dependence of optimum coagulation dose on the concentration of colloidal material.

The aggregation of colloidal particles can be considered as involving two separate and distinct steps:

(1) particle transport to effect interparticle contact, and (2) particle destabilization to permit attachment when contact occurs. Theories of particle transport are based on fluid and particle mechanics; theories of particle destabilization are based on colloid and surface chemistry. The design of structures and flocculation equipment for a coagulation process is influenced by a consideration of interparticle contacts; the selection of the type and dosage of coagulant is based on a consideration of particle destabilization. The design of the overall coagulation process must provide for both of these steps.

Present understanding of particle transport in coagulation processes is based on the work of Smoluchowski. When the interparticle contacts are produced by Brownian motion the transport process is some times termed perikinetic flocculation. When contacts between particles are caused by fluid motion the process is sometimes termed orthokinetic flocculation (2,18).

2.1.1 Mechanism of Coagulation

The process of coagulation has progressed from the art of the early days attempts to purify drinking water to today's sophisticated techniques, equipments and studies of many exotic coagulants for accomplishing the same objective on a much larger scale.

There has been two main approaches in the field of colloid science, to explain the basic mechanism involved in the stability and instability of colloid system. The first or the so-called chemical theory assumes that colloids are aggregates of definite chemical structural units, and coagulation of colloids is the result of a precipitation of insoluble complexes that are formed by specific chemical interactions. The second or the physical or double layer theory emphasizes the importance of the electric double layers surrounding the colloidal particles in the solutions and the effect of counter-ion adsorption and zeta potential reduction in the destabilization of colloidal systems.

2 1.2 Physical Mechanism : Electric Double Layer

The colloidal particles in a solution develop electrical charges on the particle water interface. The origin of these charges may be due to imperfections within the crystal lattice of the particle (isomorphic replacement), the dissociation of the ionizable groups of the colloid itself or to the adsorption of low-molecular weight ions onto its surface (4). As a result of this charge development a charge balance must be established in the vicinity of colloidal particle to fulfil the requirement

of electroneutrality. Helmholtz considered the picture of the charge balance as two surface charges separated by a constant distance as a simple condenser in a solution. The charges on the particle surface formed either the positive or negative portion, whereas the opposite charges (counterions) in solution comprised the other portion. This over simplified model was latter improved by Gouy by introducing the concept of the diffused double layer to which Chapman applied Poisson's equation to find the equilibrium distance of ions in the double layer. Ultimately stern suggested an electrical double layer that combined the Gouy-Chapman diffuse layer and the Helmholtz fixed layer. In this Stern-Gouy, diffused, double-layer model part of the counter-ions remains in a compact, stern layer on the charged colloid surface as a consequence of the existence of strong electrostatic forces as well as Van der Waals forces. The other part of the counterions extends into the bulk of the solution and constitutes the so-called Gouy-Chapman diffuse layer (1,2,4).

Depending on the characteristics of different types of counter-ions involved in the colloidal systems, the repulsive zeta potential of the particle can be reduced

in the following way (15,16) (a) by the compression of the double layer thickness due to incorporation of simple counterions into the diffuse double layer; or (b) by the specific adsorption of the counter ions on to the particle surface, with a concurrent reduction in the surface potential of the colloidal particle. Coagulation by the first type of zeta potential reduction is called the double layer interaction mechanism and the second type of coagulation is termed as specific surface interaction mechanism. The coagulation of a colloidal system with simple monhydrolysed ions (such as sodium and calcium ions) is due to double layer interaction. The specific surface interaction phenomena can be fully demonstrated by a charge reversal behaviour of colloidal particles in presence of highly charged, low molecular weight polymeric species (such as the hydrolysis products of aluminium and iron ions).

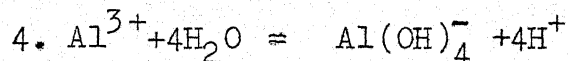
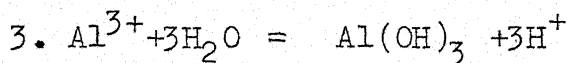
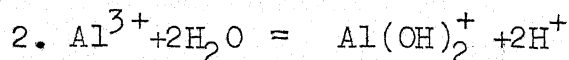
2.1.3 Chemical Mechanism : Metal ions

Hydrolyzing coagulants (such as aluminium and iron salts) have been used for centuries in water purification. Theories as to the nature of the interaction with various colloidal impurities have changed over the years from the early concept of simple enmeshment by the gelatinous hydroxides to invocation of the Schulze-Hardy rule, followed

by emphasis upon the properties of hydrolysis products, and finally, specific chemical interactions and various adsorption model (14,17,18).

The hydrolysis of polyvalent metal ions in aqueous solution has been studied in detail by many investigators, with considerable attention devoted to those ions of interest in coagulation.

The hydrolysis of iron (III) and aluminium (III) ions to yield a variety of hydrolysis products may be presented using Al^{3+} .



Polymerization of some of the above to yield polynuclear species have been postulated by many investigators (1,2,4) to explain solution properties. The most widely accepted general form are the complexions, such as

$Al_x(OH)_{2.5x}^{0.5x+}$, the species $Al_8(OH)_{20}^{4+}$; $Al_7(OH)_{17}^{3+}$ and $Al_{13}(OH)_{34}^{5+}$ Stumm and O' Melia (17) presented a simple adsorption model to explain coagulation stoichiometry and they pointed out that "Coagulation phenomena in natural systems are quite specific. This specificity arises from the fact that colloid stability is affected by Colloid-solvent, coagulant-solvent, and colloid-coagulant interactions".

The double layer model which considers only one type of colloid coagulant interactions (Coulombic forces) and neglects solvent interactions with the colloid and the coagulant, therefore can be used to describe only a very limited number of systems. It is difficult if not impossible to find a natural system that can be characterized by this model. Over emphasis on electrostatic phenomena in studies of coagulation in natural systems can produce results that are inefficient, uneconomical or both.

2.2 Polyelectrolytes

A polymer molecule is defined as a series of repeating chemical units held together by covalent bonds. Special classes of polymers, possessing ionizable functional groups along the polymer chain, are called polyelectrolytes or polyions (19) when these groups dissociate,

the polymer molecules become charged either positively or negatively depending on the special functional groups present, and are referred to as cationic and anionic polyelectrolytes respectively. Polyelectrolytes possessing no ionizable functional groups are termed as nonionic. A large variety of cationic, anionic and non anionic polyelectrolytes have been approved by the US Public health service (20) for use in water treatment.

2 2.1 Natural and Synthetic Polyelectrolytes

Most of the natural polymers and their derivatives used as coagulant aids are based on a polysaccharide skeleton and have anionic properties derived from the presence of carboxyl groups. The molecular weight of these materials may be up to 10^5 . Not much is known about the flocculating properties of Proteinaceous substances such as gelatins and glues, and these have not yet found any significant use in water treatment.

A large number of synthetic polyelectrolytes are commercially available, ranging in molecular weight from 10^4 to 10^7 . The exact formulation of any of these products is a matter of commercial secrecy. One advantage that natural polymers have is that they are nontoxic (21).

2.2.2 Theory of Action of Polyelectrolytes

In considering the flocculating action of polyelectrolytes it has been necessary to put forward a completely different mechanism based on interparticle bridging by the long polyelectrolytes chain. It is highly significant that many mineral suspensions carrying a net negative charge can be flocculated readily by anionic polyelectrolyte and it is difficult to explain this on the basis of classical theories of coagulation.

LaMer (22) postulates that the polymer molecules attach themselves to the surface of suspended particles at several adsorption sites, portions of the macromolecule extending into the bulk of solution. Bridging takes place as a result of effective contacts between polymer segments and vacant bonding sites on other particles. The total process may be regarded as a very rapid adsorption followed by a slower bridging process. If the polymer concentration is increased then the number of vacant sites is decreased until eventually a situation is reached where the vacant sites are so sparse that the suspension becomes stabilized. Michaels (25) have stated that "adsorption of polyelectrolyte on the particles does not necessarily occur at ionizable groups, the main purpose of which is to cause extension of

the polymer chain by mutual repulsion". He postulated that adsorption of polyelectrolytes on clays was probably effected by hydrogen bonding through amide and hydroxyl groupings. It is clear, however, that when cationic polyelectrolytes are used to flocculate negatively charged mineral suspensions the adsorption of the polyelectrolyte must be influenced by Coulombic attraction.

In the study of interactions between synthetic polyelectrolytes and dilute kaoline suspensions, Black and others (26) showed that the electrophoretic mobility of the particles was reduced considerably by small additions of cationic polymer. Higher concentrations of polymer were necessary for optimum flocculation. It was concluded that cationic polymers acted first as a coagulant in reducing the forces of repulsion between clay particles and then as a flocculant. The clay could also be flocculated by a strongly anionic polymer if a sufficient concentration of calcium ions are present. It was concluded that the function of calcium was first, to reduce repulsive forces between the clay and polymer so that polymer adsorption could take place, second, to reduce the forces between clay particles so that they can get close enough for bridging to occur; third, to reduce interactions between extended segments of absorbed polymer molecules

thereby facilitating the bridging operation. The magnetude of these effects depend on the number of anionic groups of the polymer molecule.

Several authors including LaMer (22) have drawn attention to the fact that the degree of mixing and the mixing time may be critical inflocculation in the polyelectrolytes. It has been stated that three cardinal rules for the proper use of a high molecular weight polyelectrolyte are : (1) Addition of polyelectrolyte in the form of a very dilute solution (0.05% or less); (2) Use of multiple point addition to ensure contact with every portion of the system; and (3) The application of mild agitation at the addition points and shortly thereafter to assist in the dispersion of the polyelectrolyte in the process stream.

2.3 Bioflocculation

Bacteria and other micro-organisms such as viruses, algae, and protozoa, can be considered to be hydrophilic biocolloids (27). They carry a net negative charge (ZP-12 to -40mv)(38) within the pH range of interest. The stability of dispersion of hydrophilic biocolloids depend on the forces interacting between the particles themselves as well as on the forces interacting between the particle

and water. Hence electrostatic repulsive forces do not explain fully the stability of bacterial suspensions. Bacterial suspension can form stable suspension even at their isoelectric point. Thus, the concept of zetapotential (i.e. opposing effects of repulsive interactions between electric double layers and of attraction by **Vander Waal's** forces) alone can not account satisfactorily for the phenomenon of bioflocculation. The interaction of the hydrophilic surfaces with the solvent (water) also is an important stability factor.

Colloid chemical investigations on the flocculation of Bacteria are made difficult by the great variability of the bacterial surface composition. Proteins, lipids, polysaccharides, and nucleic acids. Combine in various proportions and occur on the surface in the form of cell walls, capsules, flagellae, fibrils, and filaments of various kinds. The unhomogeneity of the microbial population encountered in the waste treatment systems represents additional difficulties for a simple interpretation of observed facts.

The external surface of bacteria essentially consists of amphoteric and hydrophilic groups. It acquires its charge through acid-base interactions of the functional

ionogenic groups. Thus, the surface charge density of bacteria is strongly pH dependent. Adsorption of simple ions to the surface is limited by its hydration and is less important in establishing the surface charge density. The isoelectric points of bacteria are at very low pH values (pH 2-4) and are significantly lower than that of protein (pH 5-8). This suggests the occurrence on external surfaces of substances that have lower isoelectric point than protein such as glutamic acid, lipids, glucose and mucopolysaccharides.

Boyle, Crabtree and others (28) have proposed six flocculation mechanisms. But uncertainty exists in the natural bioflocculation in biological waste treatment. Busch and Stumm (29) have interpreted the biological self flocculation in terms of naturally produced polyelectrolytes which form the bridges between the individual bacteria. McGregor and Finn (30) have stated that "the mechanism of cell flocculation by chemical additions appears to be a highly complex phenomenon. Many of the factors affecting flocculation are related to the presence of biological polymers released by the cell. So the chemical flocculation of microorganisms can be interpreted similarly in terms of a polymer bridge model.

Bacteria and microorganisms are a complex bio-colloidal systems. These usually have negative charge in the pH ranges encountered in surface waters. The flocculation is made difficult because most of these are likely to be hydrated (solvated) and as such offer resistance to either coagulant or the polymer causing coagulation and flocculation. Studies with several genera of bacteria indicate that the mechanism of flocculation is highly complex. Such interacting variables as temperature, ionic environment, physiological age, flocculant, bacterial genus, and surface shear have been observed. Jar test experiments with washed cells indicate that many of the variables are related to the release by the cell of proteins, nucleic acids or polysaccharides. When released, these polymers may increase the required dosage of flocculant for recovery as in the case of E.coli, or the dosage may decrease as it does for Lactobacillus (30).

2.4 Nirmali Seed in Water Clarification

Nirmali tree or Strychnos potatorum is one of the group of trees which produce the complex and related alkaloids, strychnine and brucine. It belongs to the family of Longaniaceae and grows profusely in various parts of India and South East Asia. The seed of this tree is

broadly lenticular and has a dirty whitish grey colour. It is about half an inch in diameter and a quarter inch in thickness. The seed has long been recognised for its medicinal properties. A paste made from nirmali seed precipitates suspended siliceous impurities (32) in raw water. Subbaramaiah and Sanjiv Rao (33) found from electrophoretic measurements that the paste carried a weak negative charge. These authors studied the action of strychnine and albumin on synthetic turbid water and concluded that coagulation of turbidity by the paste is due to the presence of these two ingredients. Hasker and Khendukar (34) have reported the presence of complex polysaccharides in the seed containing galactomannan residues, similar to those found in guar gum. Sen and Balasu (7) showed the extract of nirmali seed to be effective as coagulant and coagulant aid in producing well defined flocs with rapid settling characteristics for all pH values for naturally occurring turbid water. The pilot plant studies carried out by Bulusu and Sharma (8) conclusively proved the efficiency of Nirmali seed extract as an aid to alum. Dhekane and others (9) found out the optimum pH and dose of nirmali seed and concluded that this can be used mainly as coagulant. They showed the

non toxicity of the extract and tried its chloroderivatives in the removal of bacteria. Rao and Sastry (10) also concluded it to be a coagulant aid in turbidity and colour removal.

McGarry (42) tried with the extract as coagulant aid with alum in the removal of algae, but he did not report the results.

Most of the above workers have studied the use of Nirmali seed extract either as coagulant or coagulant aid but none of them mentioned about the mechanism of removal.

CHAPTER III

SCOPE OF THE PRESENT STUDY

The selection of the optimum type and dosage of coagulant can not be made for any water or waste water without experimental analysis. Theories of colloid destabilization are just not sufficiently developed at present to permit prediction such of a selection without some experimentation. It is doubtful if colloid chemical theories will ever be able to do so (2).

There are many factors which influence coagulation. Among them may be listed (1).

- (1) Type of coagulant
- (2) Quantity of coagulant
- (3) Amount and character of colour and turbidity
- (4) Other chemical characteristics of water
- (5) Hydrogen ion concentration (pH) of water.
- (6) Time of mixing and flocculation
- (7) Temperature of water
- (8) Intensity of agitation
- (9) Presence of nuclei

The most important single test for the determination of optimum coagulant dosage is the "jar test". The equipment for this test and the directions for its proper performances have been described by numerous investigators including Cohen (31).

It is difficult to control all the variables in water and waste water coagulation. In the present study effect of dose of coagulant, pH, and original turbidity were seen on the removal efficiencies..

Previous work shows the efficiency of nirmali seed extract as coagulant and coagulant aid in the removal of turbidity, mostly of inorganic nature from natural water.

Present study is devoted to the exploration of its potentiality of removal of organic turbidity due to bacteria and algae. With this end in view the jar tests were conducted with the following samples to see the effectiveness of Nirmali seed in removing organic turbidities.

- (1) Controlled *E.coli* suspension
- (2) Synthetic turbidity with kaolin
- (3) Mixed suspension of *E.coli* and kaolin
- (4) Natural surface water

(5) Oxidation pond effluent

(6) Raw sewage

Plate counts of supernatant were done for the sample numbers 3 and 4 to see its effectiveness in bacterial removal. Tests with synthetic samples were conducted to study its effectiveness in controlled condition and then with natural surface water to see its applicability in the field.

Surface tension measurements were also conducted to study the mechanism of removal.

CHAPTER IV

MATERIALS AND METHODS

4.1 Materials

The following chemicals have been used in the preparation of various reagents and media to carry on the experiments.

4.1.1 Chemicals Required For The Characterization of Nirmali Seed

1. Phenol (BDH)
2. Sulphuric acid (BDH)
3. D-Glucose (BDH)
4. Sodium Carbonate (BDH)
5. Copper Sulfate (BDH)
6. Solvent Ether
7. Sodium Potassium tartrate
8. Bovine serum albumin
9. Folin ciocaltoin reagent
10. Nirmali seed extract

4.1.2 Biological Media Culture Medium for E.Coli (A 19)

Bactrotyptone (Difco)

10 g

Yeast extract (Difco)	5 g
Sodium chloride (BDH)	10 g
D-Glucose	1 g
1 N NaOH	2 ml
1 N Ca Cl ₂	2 ml
Distilled Water	1 litre

Medium for Plate Count

Beef extract	3 g
Peptone	5 g
Agar	15 g
Distilled Water	1 litre

Selective Medium : EMB Agar

Peptone	10 g
Lactose	5.0 g
Sucrose	5.0 g
K ₂ HPO ₄	2 g
Eosin y	0.4 g
Methylene blue	0.06 g
Agar	15 g
Distilled Water	1 litre

4.1.3 Other Materials

1. Na₂ HPO₄ and Na H₂ PO₄

2. $\text{Ca Cl}_2 \cdot 2\text{H}_2\text{O}$
3. Kaolin
4. Nirmali seed powder
5. $\text{H}_2 \text{SO}_4$
6. Na OH
7. $\text{Al}_2 (\text{SO}_4)_3 \cdot 16 \text{H}_2\text{O}$: Alum
8. Na HCO_3
9. Water · Distilled water, tap water
and canal water
10. Glass ware
11. I.I.T /K Campus Oxidation pond effluent
12. I.I.T./K Campus Raw Sewage

4.1.4 Use of Material

The chemicals used for the preparation of reagents for the characterization of Nirmali Seed were as mentioned by Whistler (35) and Litwack (36).

Biological Media for E.Coli A 19 was prepared as mentioned. Nutrient Agar for plate count and EMB agar were prepared as mentioned in standard methods (37).

E. coli used in the study were originally obtained from the Environmental Engineering Laboratory, University of Illinois, Urbana, IU, U.S.A.

Sodium monohydrogen phosphate and di-hydrogen phosphates were used for the preparation of buffers at pH 6, 7, and 8 as described by Plummer (39).

Kaolin was used to prepare stock turbid suspension as per standard methods (37).

0.1N H_2SO_4 and 0.1N NaOH were used for pH adjustment. $NaHCO_3$ was used for alkalinity adjustment.

Nirmali seed powder was used for the preparation of nirmali seed extract.

Tap water was used for general washing where as distilled water was used for washing and preparation of reagents. Lower Ganga Canal Water was used as natural turbid water. The characteristics of which is given below in Table - 1.

TABLE 1
PROPERTIES OF CANAL WATER

Properties	Minimum	Maximum
Temperature	20°C	30°C
pH	8.0	8.4
Total alkalinity(mg/l as $CaCO_3$)	120	190
Total hardness (mg/l as $CaCO_3$)	110	160
Turbidity FTU	30	500

Glassware : The glasswares were soaked over night in 0.3 percent B-300 Teepol (manufactured by surfactants Pvt. Ltd. for National Organic Chemical Industries Bombay-18) followed by rinsing with tap water and distilled water. Pipettes were washed in automatic pipette washer and finally these were rinsed with distilled water. Subsequently glass ware was sterilized in hot air oven at 180°C for 2 hours or longer as required by Standard Methods (37).

4.2 Apparatus

The following apparatus were used to carry out the experiments

1. Laboratory stirrer (Phipps and Bird Inc. Richmond, Va. USA) was used for conducting jar test experiments.
2. Elico pH meter model LI-10 (Electronic and Industrial Instrument, Co. Pvt. Ltd. Hyderabad, India) was used for pH measurement.
3. Bauch and Lomb spectronic-20 colorimeter/spectrophotometer (Baush and Lomb Co. Rochester, N.Y. 14602) was used for optical density measurements.
4. Hellige Turbidity meter (Hellige, Inc. 877 Stewart Ave garden city, N.Y.) was used for turbidetric measurements in FTU.

5. Sorvall centrifuge (Ivan Sorvall, Inc. Norwalk, Conn, U.S.A) was used for the preparation of bacterial pellets.
6. Air oven (Marang Pvt. Ltd. Bombay, India) was used for sterilizing glass ware.
7. Water bath (Gansons Pvt. Ltd. India) was used for keeping the media at 45°C.
8. Coloney counter made in India was used for counting the bacterial colonies.
9. Zetameter (M/S. Zetameter Inc. N.Y. U.S.A.) was used for zeta potential measurements.
10. Perkin Elmer 521.
(Perkin Elmer Corp. Norwalk, Conn., U.S.A.)
was used for I.R. Spectral study.
11. Cenco Dunouij Interfacial tensometer (Cat. No.70549) was used for surface tension measurements.

4.3 Experimental Techniques

4.3.1 Preparation of Extract

1.000 gm of Nirmali seed powder was taken in 100 ml of distilled water and blended for 10 min at high speed in a food blender. This volume was made upto 1000 ml and 0.5 ml of HCl was added to preserve it against

bacterial decomposition. The extract was ready for use and was stored in refrigerator.

Nirmali Seed powder was supplied by Mr. N.Y.Dhekane of Maharashtra Engineering Research Institute, Nasik.

4.3.2 Characterization of Nirmali Seed

(a) Carbohydrate Estimation

Carbohydrate estimation was done by phenol sulphuric acid method (35). In the presence of conc. sulphuric acid, sugars are hydrated to form furfural derivatives which yield yellow orange colour on complexing with phenol. The method can be used for sugars, polysaccharides, methyl derivatives and uronic acids. This method was selected as it is simple rapid and sensitive.

Sugar (glucose) solution containing 0-70 g was taken in different test tubes and the volume was made upto 1 ml. 1 ml of phenol solution (5g phenol in 100 ml of water) was added and mixed thoroughly. From a fast flowing pipette, 5 ml of conc. sulphuric acid was added to each tube in such a way that stream hits the liquid surface directly. After 10 minutes, tubes were shaken and kept in a water bath (25-30°C) for 20 min. Optical density of the yellow orange coloured solution was measured

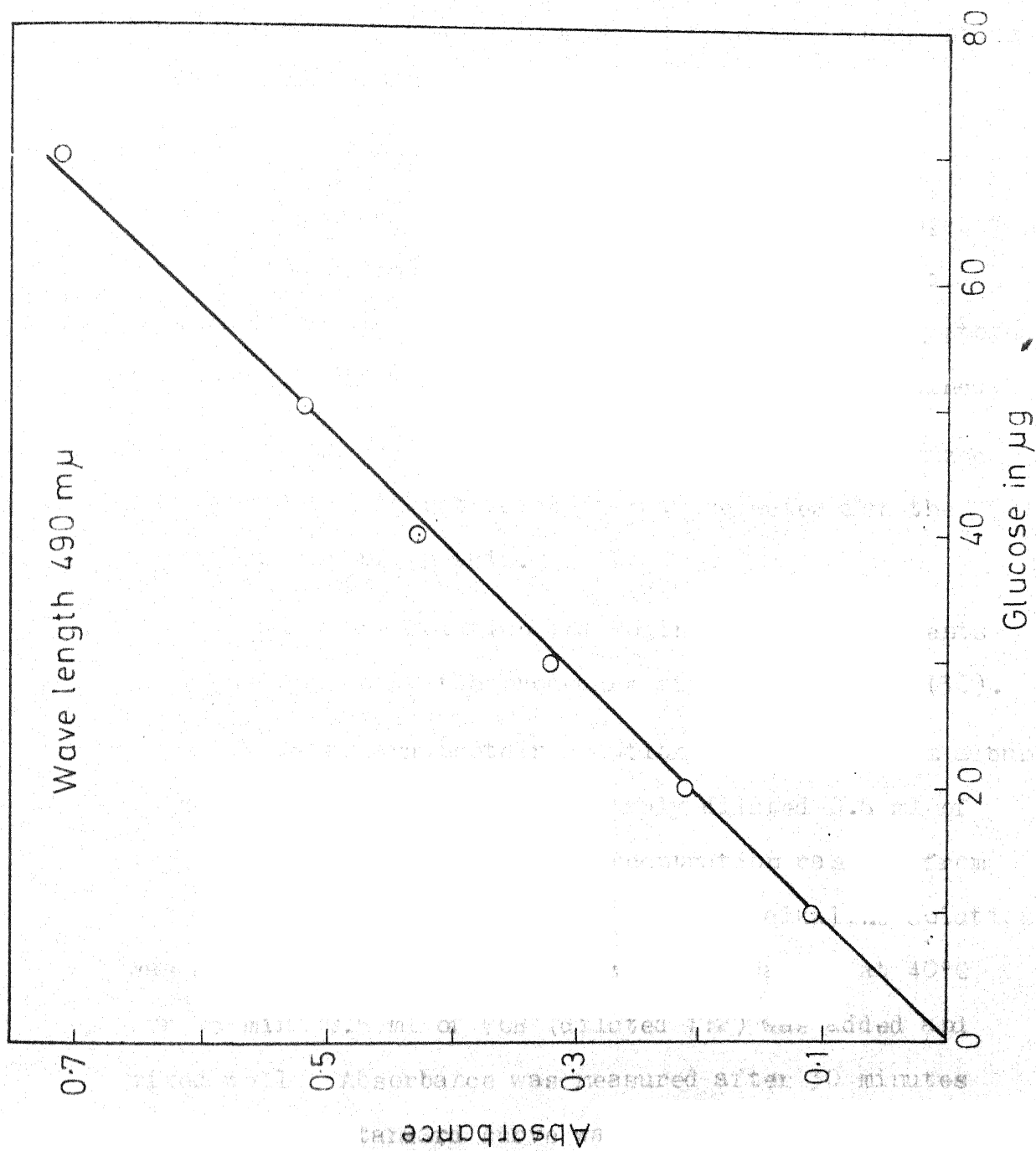


FIG.1 STANDARD CURVE FOR DETERMINATION OF CARBOHYDRATE

at 490 m μ on spectronic 20 (1 cm light path) and a standard curve was prepared (Fig. 1). Identical procedure was followed for determining the carbohydrate content in Nirmali Seed extract.

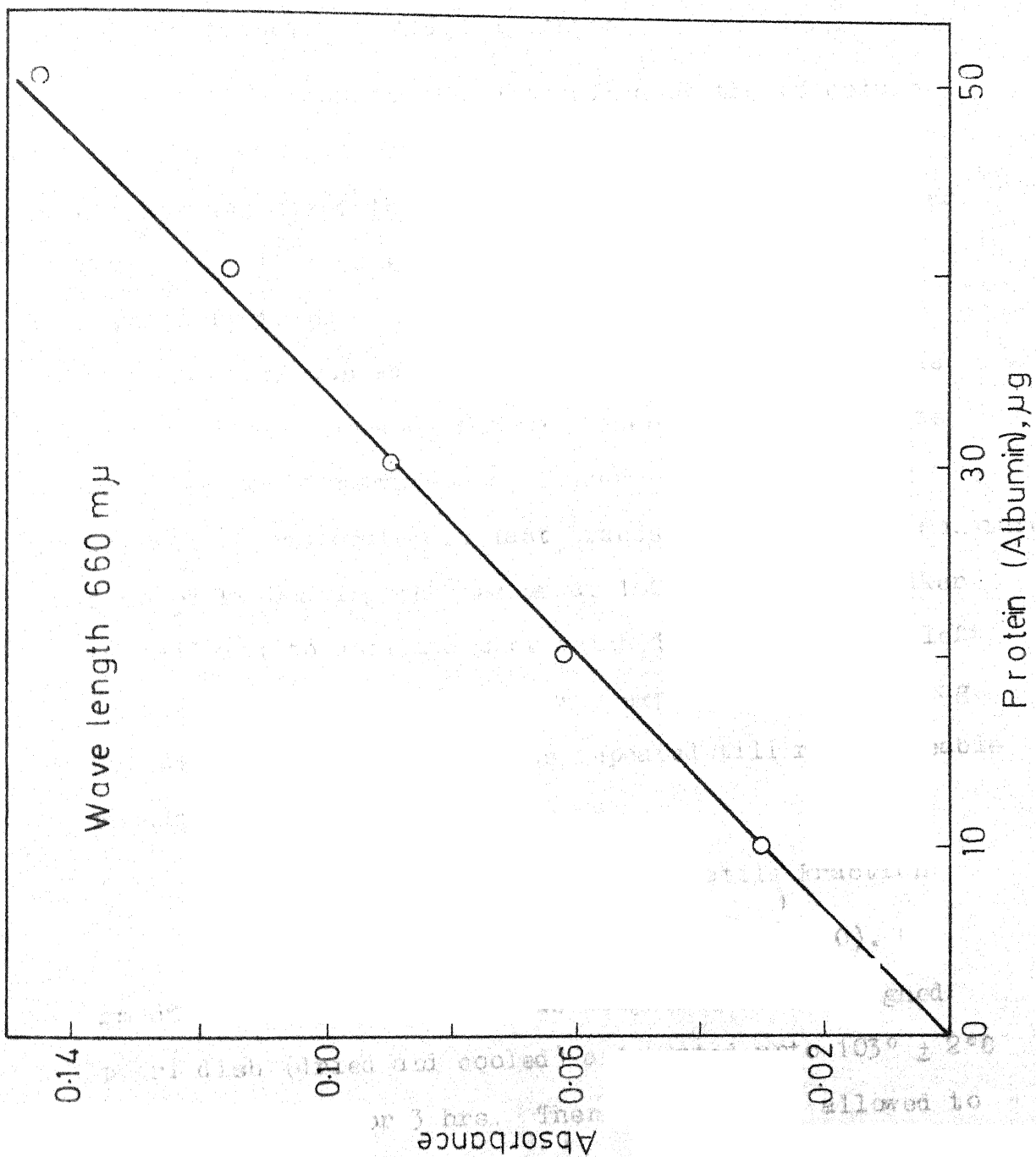
(b) Protein Estimation

Due to biuret reaction of protein with copper ion in alkaline medium and reduction of phosphomolybdic phosphotungstic acid in the Folin's reagent by tryptophan and tyrosin present in protein blue colour is obtained.

Folin Ciocalteu reaction, being 100 times more sensitive than biuret reaction, was selected for the determination of protein.

Alkaline solution and Folin Ciocalteu reagents (FCR) were made by the procedure given by Litwack (36).

A standard protein solution of bovine serum albumin (1 mg/ml) was prepared and suitably diluted 0.5 ml of protein solution was taken (concentration ranging from 0-100 μ g/ml) in several tubes. 5 ml of alkaline solution was added to each tube, mixed well and warmed at 40°C for 15 min. 0.5 ml of FCR (diluted 1:2) was added and mixed well. Absorbance was measured after 30 minutes at 660 m μ . Standard curve was prepared for protein



estimation (Fig. 2). Identical procedure was followed for the protein determination in Nirmali seed extract.

(c) Determination of Lipid Content

Lipid content was determined by the procedure given by Peach and Tracey (40) 50 ml of Nirmali seed extract was mixed thoroughly with 25 ml of solvent ether. Water and ether layers were separated by means of a separatory funnel. Again in the water extract portion solvent ether was added and mixed and separated. This procedure was repeated thrice. Then ether extract was transferred to a preweighed beaker. The solvent was allowed to evaporate and last traces of solvent and moisture removed by heating the beaker at 100-105°C. The beaker was allowed to cool and then weighed. The material left in the beaker was lipid. It was expressed in percentage by weight. The procedure was repeated till reproduceable results were obtained.

(d) Determination of Moisture and Volatile Fraction

Procedure given by Peach and Tracey (40). One gm of Nirmali seed powder was weighed in a preweighed petri dish (dried and cooled) and heated upto $103^{\circ} \pm 2^{\circ}\text{C}$ in an air oven for 3 hrs. Then the dish was allowed to cool and is reweighed. The process is repeated with one

hour periods until the loss in weight between two successive weighings is 1 mg or less. Cover of the petri dish was kept slightly open during heating.

(e) Determination of Ash Content

A nickel crucible was cleaned and dried up to a constant weight. 1.000 g of Nirmali seed powder was taken in the crucible and burnt for six hours under full heat of the burner. The cover was kept slightly open. With the help of tongs crucible was kept in a dessicator for cooling and reweighed. The process was repeated with one hour heating until the loss in two successive weighings was one mg or less.

The results of the test of characterization of Nirmali seed powder were collected and given in Table 2.

TABLE 2

Constituents of Nirmali Seed

Items	Weight Percentage
Carbohydrate content	52.5
Protein content	16.3
Lipid content	9.0
Moisture and volatile fraction	11.5
Ash content	2.1
Other ingredients (By difference)	8.6

4.3.3 Preparation of E. Coli Suspension and Standard Curve for E.Coli Concentration

Biological medium for E.Coli A19 was prepared and sterilized. Four to five hours after inoculation (early log growth phase) bacterial pellets were prepared by centrifuging at 10,000 rpm for 5 min in a centrifuge. These pellets were resuspended in phosphate buffer (0.02M) of pH, 6.2, 7.1 and 8.0 and recentrifuged. Pellets were resuspended in phosphate buffer to have E.coli cells free of culturing medium.

By measuring absorbance at different wave lengths, optimum wave length was determined as shown in Fig. 3. At this optimum wave length absorbance of different E coli concentration were measured and plate count was done with EMB agar medium. A standard curve for E.coli concentrations per ml VS Absorbance was constructed (Fig. 4).

4.3.4 Experimental Procedures

a) Stock turbid suspension of Kaolin was prepared as per standard methods (37). Alkalinity of turbid suspension was adjusted to 200 mg/l (as Ca CO_3) by NaHCO_3 (concentration 10 mg/ml of NaHCO_3 as Ca CO_3).

b) In Kaolin turbid suspension E.coli suspension was added such that its concentration was 100/ml.

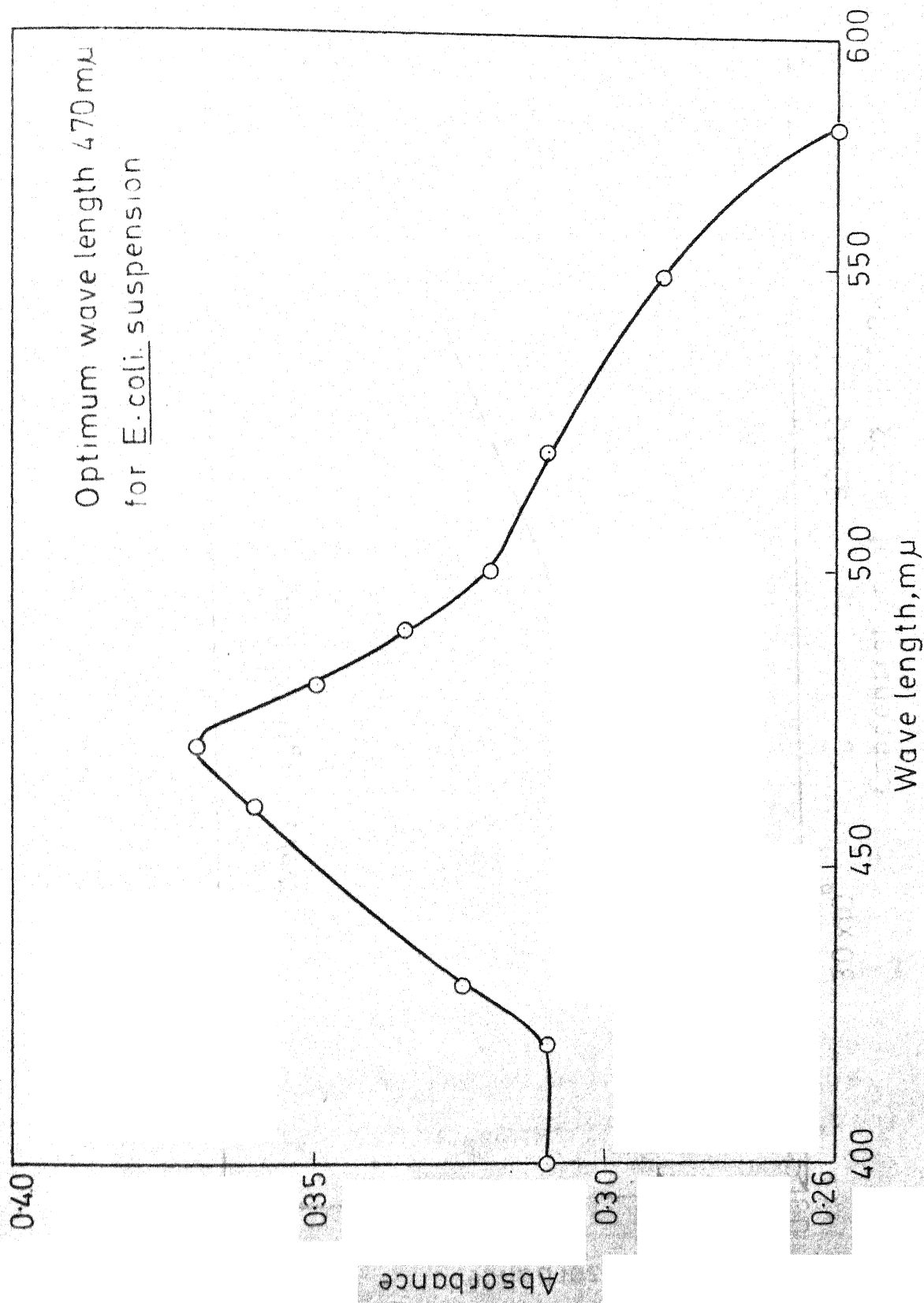


FIG.3 DETERMINATION OF OPTIMUM WAVE LENGTH FOR E. COLI-SUSPENSION

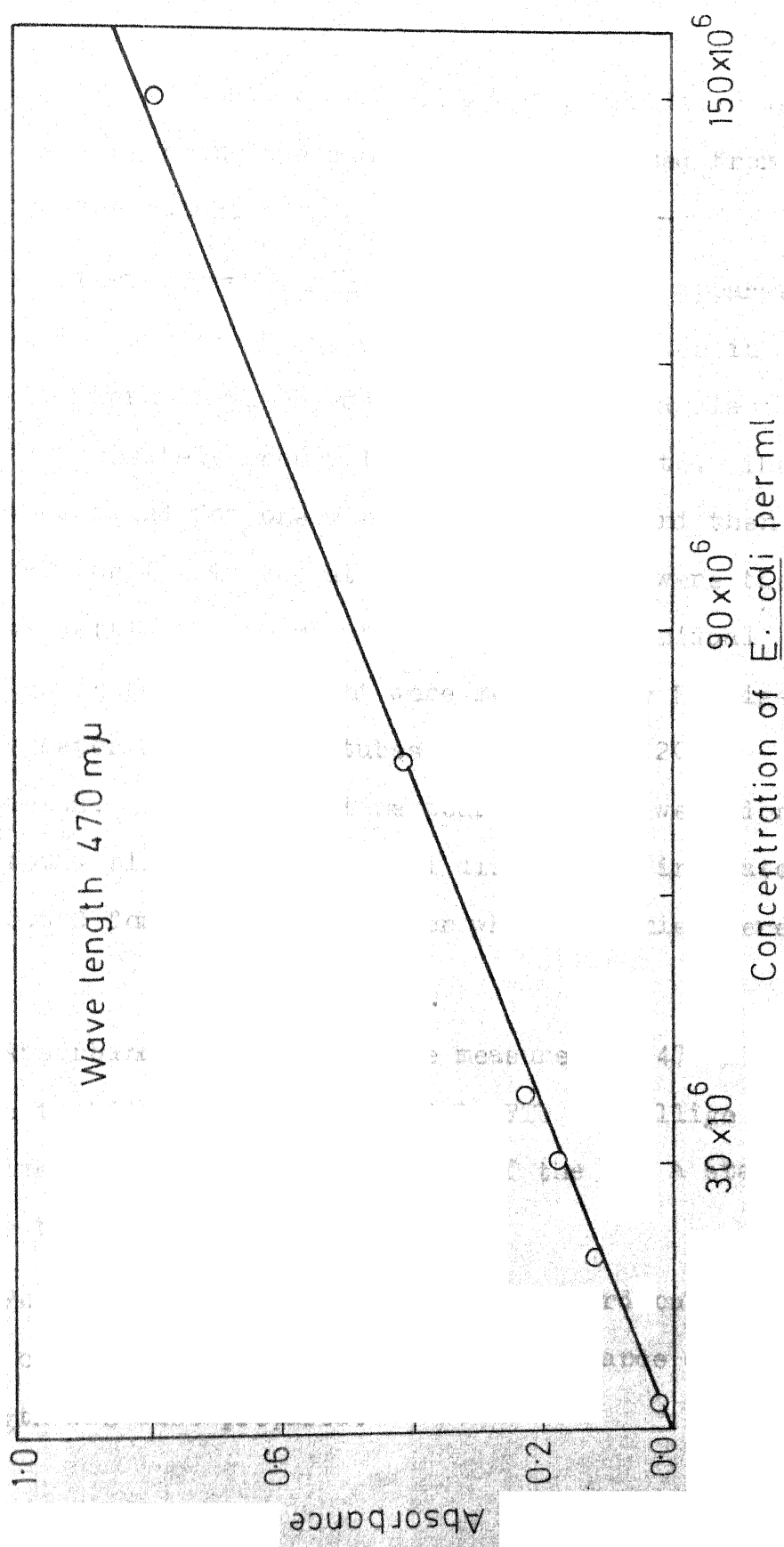


FIG.4 STANDARD CURVE FOR *E. COLI*

c) Canal water samples of different turbidities were prepared by dispersing the clay and silt obtained from the bed of the same canal.

d) Jar tests were conducted using jar test apparatus. Six one litre samples of water prepared were taken in one litre beakers to which various dosage of coagulants were added accurately from a 1 x 1/100 ml pipette. The samples were mixed for one minute at 100 rpm, and then flocculated for 15 minutes at 20 rpm. Samples were then allowed to settle for 30 minutes after which residual turbidities in the supernatant were measured by Hellige turbidity meter using glass tubes of 50 mm and 20 mm viewing depth. At the same time pour platings were done for the total plate counts. Petri dishes were incubated at $35 \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hrs. after which colonies were counted.

e) Absorbance of E.coli were measured at 470 m μ and inorganic turbidites were measured in FTU by Hellige turbudimeter. For the correlation of the two a standard curve has been prepared (Fig. 5).

f) For oxidation pond effluent a standard curve (Fig 6) of dry wt of algae (mg/l) VS absorbance at 390 m μ wave length was also prepared.

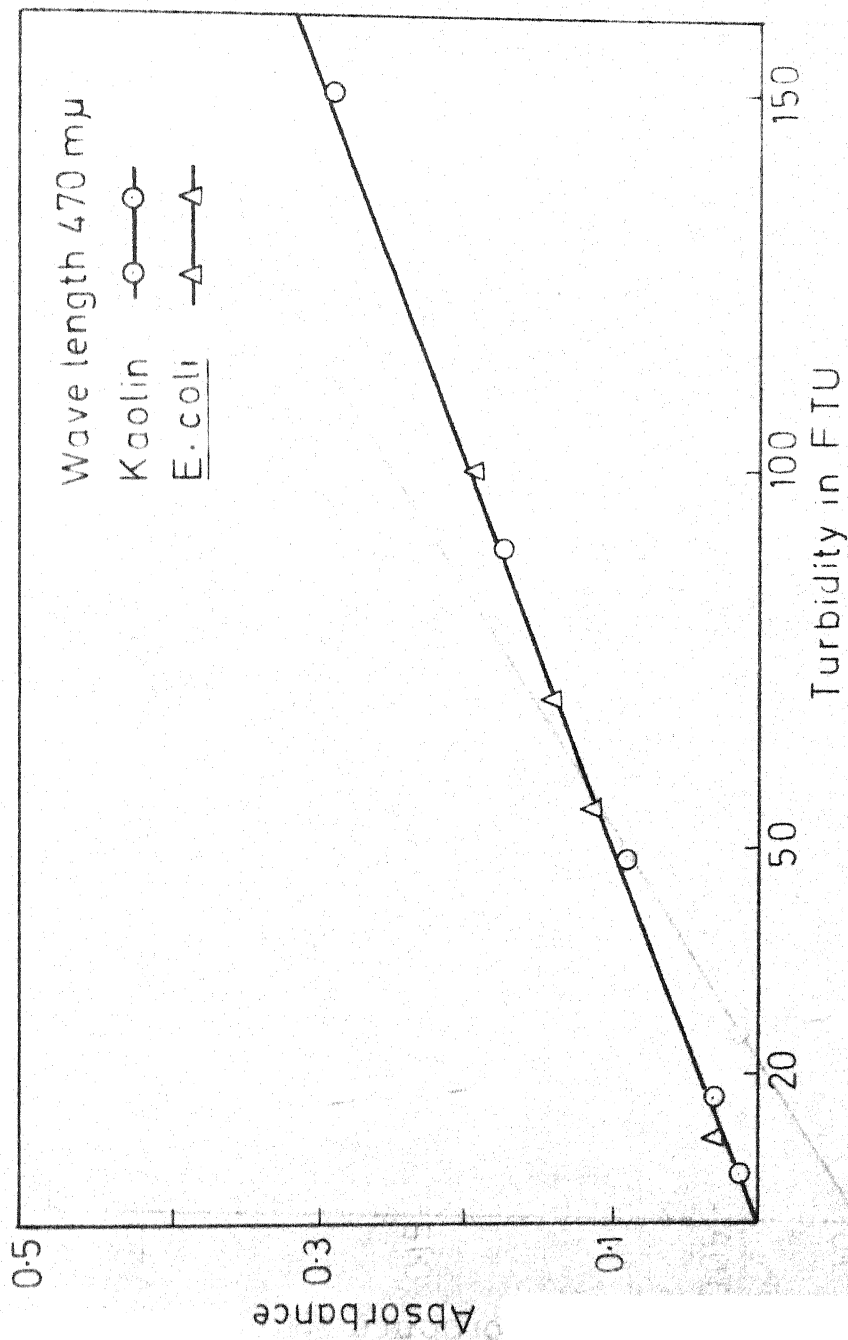


FIG.5 RELATION BETWEEN TURBIDITY AND ABSORBANCE

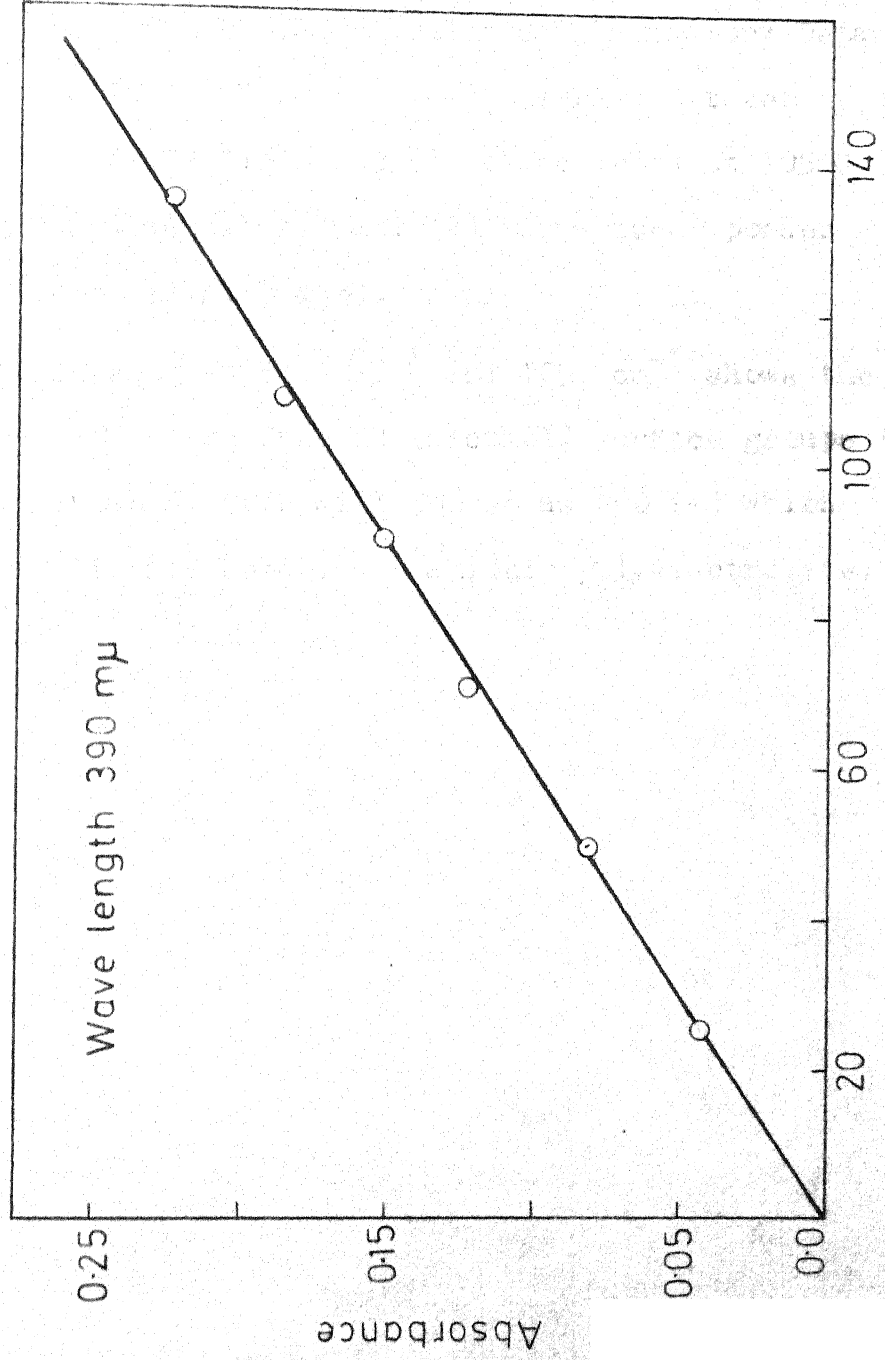


FIG. 6 STANDARD CURVE FOR ALGAE

4.4 Infra-Red Spectral Studies

I.R. Spectra from wave number 4000 to 670 cm^{-1} were obtained from the Microanalytical laboratory Department of Chemistry I.I.T Kanpur. The spectra was taken by using KBr pellets of (1) Nirmali seed powder dried at 105°C over night and dessicated and (2) Nirmaliseed powder without drying (Fig. 7 & 8).

The peaks at 3400 cm^{-1} and 1050 cm^{-1} shows the presence of (COOH and free OH (alcohol) surface groups (41) At pH more than 3, COOH will ionise as COO^{-} which shows that Nirmali seed is an anionic polyelectrolyte.

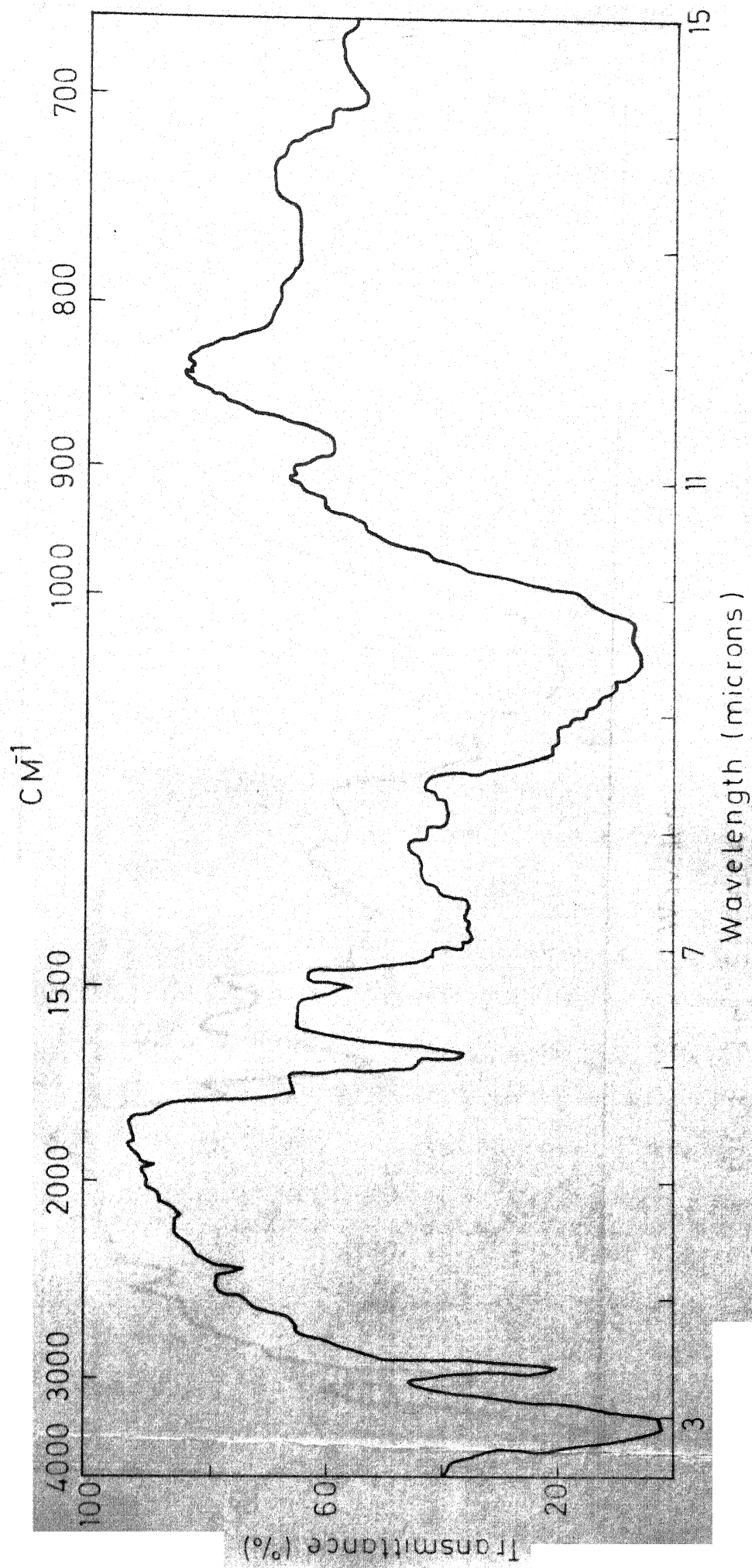


FIG. 7 I-R SPECTRA OF DRY NIRMALI SEED POWDER

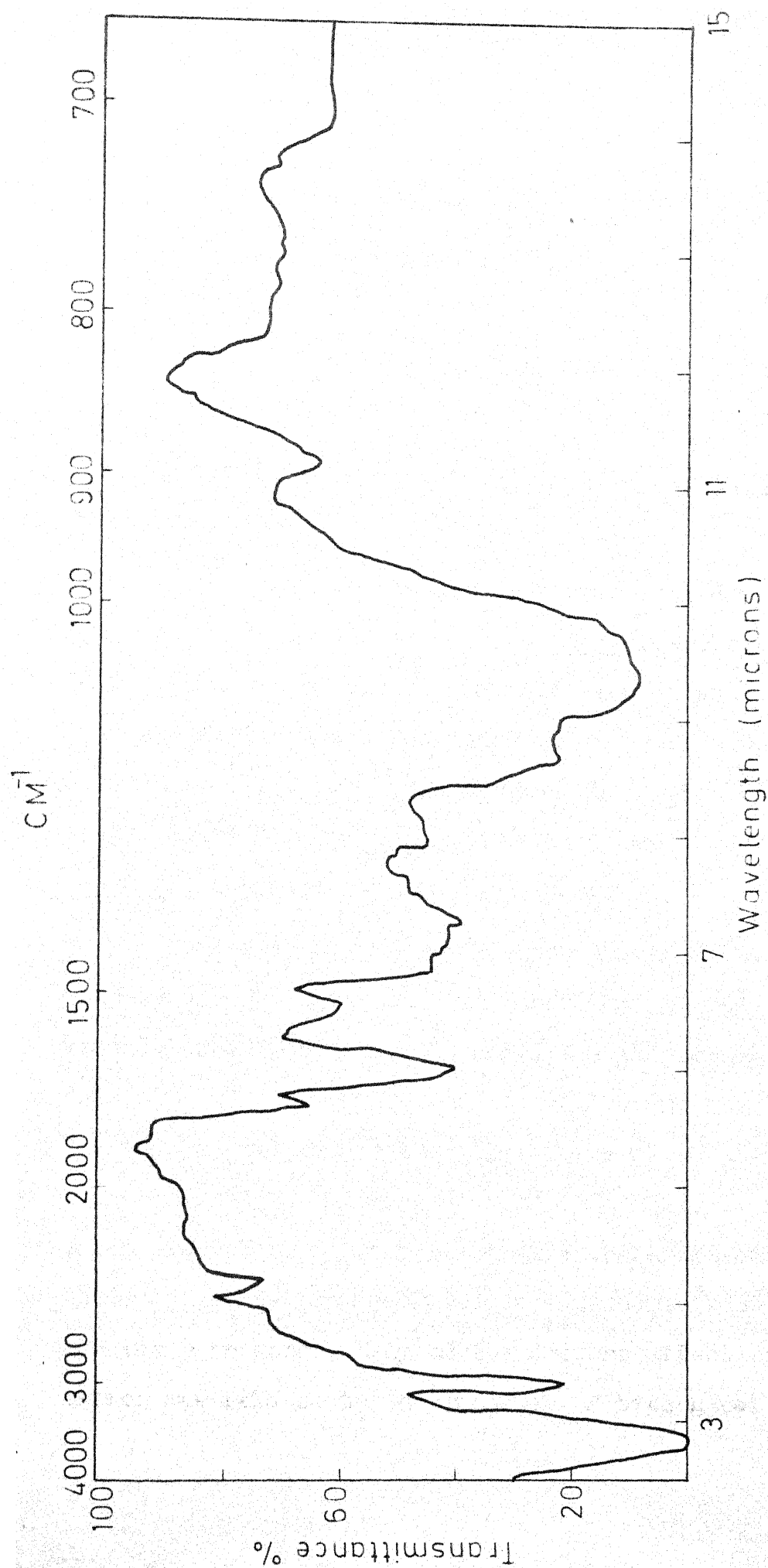


FIG. 8 I-R SPECTRA OF NIRMALI SEED POWDER

CHAPTER V

RESULTS AND DISCUSSIONS

5.1 Effect of Nirmali Seed Extract on Pure E.coli Suspension

Fig. 9 shows the jar-test data with different original concentrations of pure E.coli suspension in 0.02 M phosphate buffer. Nirmali seed extract from 0 to 100 mg/l was added at pH 6.2, 7.1, and 8.0. Absorbance measurements at 470 m μ showed that up to 10 mg/l of the extract there was no effect of the extract on E. coli suspension at pH 6.2, 7.1 and 8.0.

After 10 mg/l of extract turbidity of the suspension increased. The original concentration of E.coli suspensions were 3.7×10^7 , 5.62×10^7 , and 7.5×10^7 per ml.

McGregor and Finn (30) made a basic study of some of the factors affecting flocculation of bacteria. They used Primafloc C-7, a cationic polyamine and Baymal as the chief flocculants. They state "The mechanism of cell flocculation by chemical additives appears to be a highly complex phenomenon. Many of the factors affecting flocculation are related to the presence of biological polymers

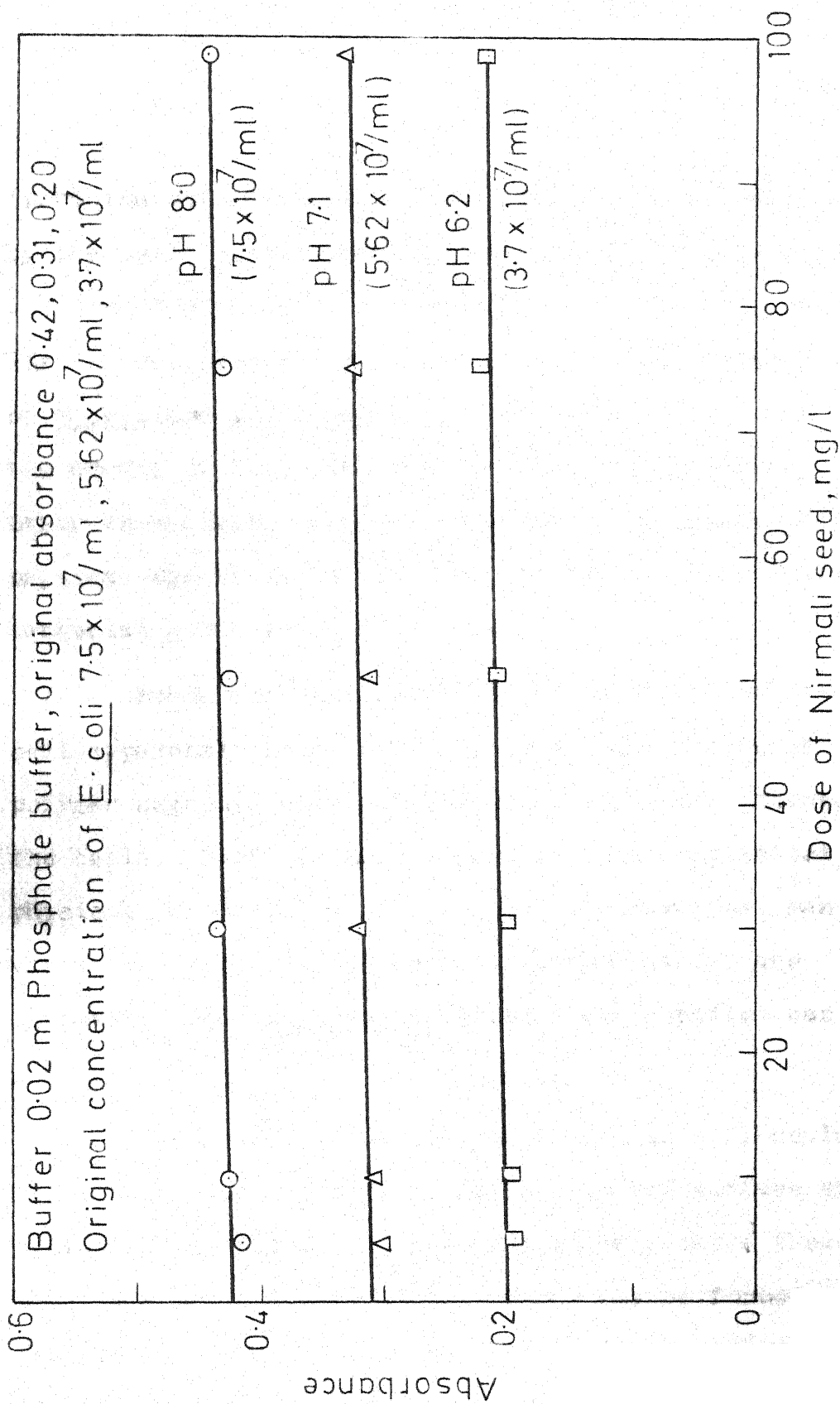


FIG.9 EFFECT OF N.S. DOSE ON E·COLI SUSPENSION AT DIFF. pH

(proteins polysaccharides, and nucleic acids) released by the cell. Their presence may either increase or decrease the amount of flocculant required for cellular aggregation. RNA and cellular proteins are released from washed cells of E. coli and P. fluorescens, and their presence increases the amount of flocculant required for flocculation. Other interacting variables observed are temperature, physical age of bacteria, type and dosage of flocculant, bacterial genus and surface shear.

Busch and Stumm (29) stated that agglomeration of cell apparently results from specific adsorption of polymer segments and from bridging of polymers between the cells. Bioflocculation results from complicated physical, chemical, and biological interactions; many variables difficult to control experimentally, are involved. No single and unifying interpretation can be given.

The ability of a polymer to act as a flocculant can depend upon its ability to bond to the surface of the colloidal particles. As a result in many cases these materials are quite specific. Bonds may be formed between particular functional groups on the polymer and specific sites on the colloidal surface. Some other

important parameters which affect the performance of a particular polymer are its molecular weight and degree of branching.

Seeing the complexity in the flocculation of micro-organisms, no unique interpretation can be given. However it may be said that (1) functional groups of Nirmali seed extract is not suitable for adsorption on E.coli cells for the reasons mentioned earlier and (2) E.coli suspension being in the early log growth phase are too stable to be flocculated. It is generally agreed that the biological suspension is easier to flocculate when it is the endogenous growth phase.

5.2 Effect of the Nirmali Seed Extract on Inorganic Turbidity

Kaolin suspension in distilled water (Alkalinity adjusted to 200 mg/l with Na HCO_3) having original turbidity 500 FTU was flocculated with the different dosages of the extract. The turbidity removal was quite effective at dose of 2 mg/l (Fig. 10). Both at higher and lower dosages the residual turbidity increases.

To be effective in destabilization a polymer molecule must contain chemical groups which can interact

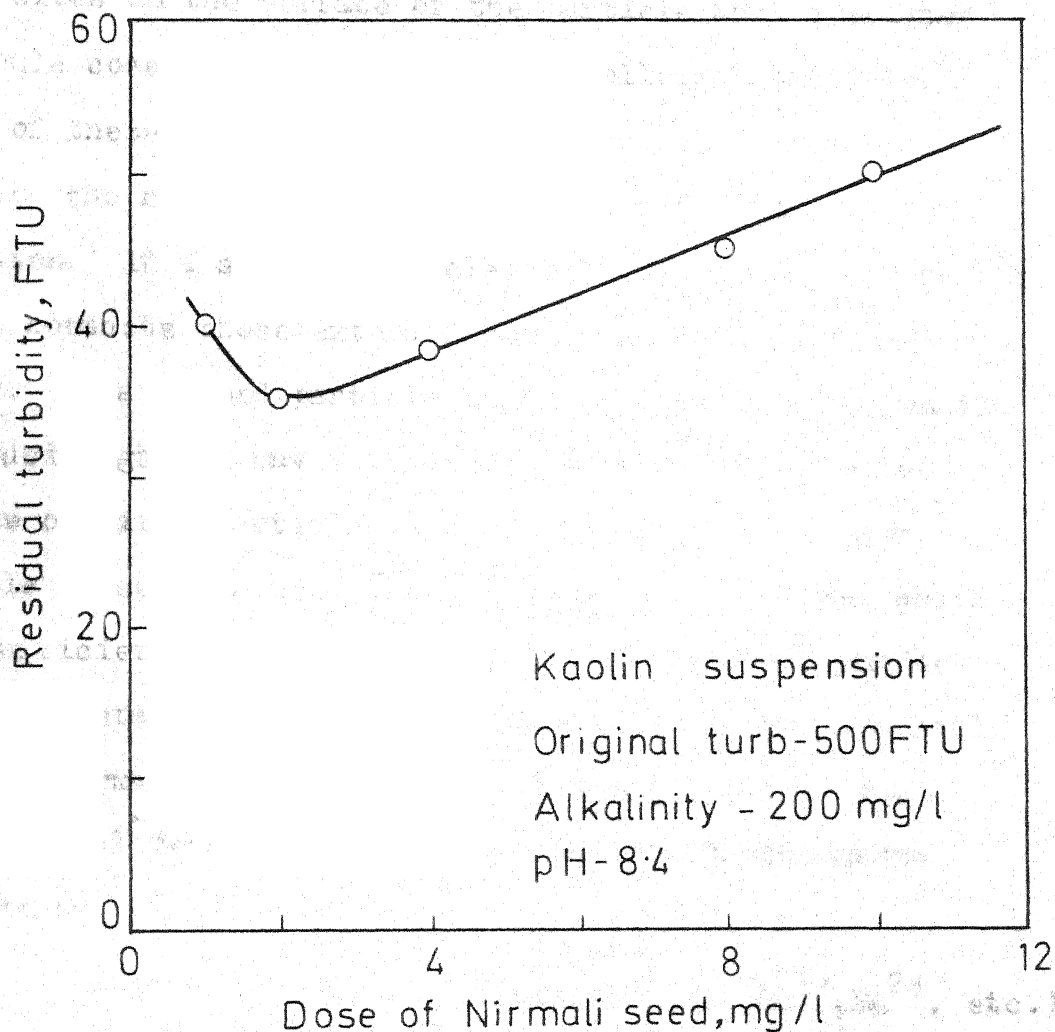


FIG.10 RESIDUAL TURBIDITY VS NIRMALI SEED DOSE FOR KAOLIN SUSPENSION

with sites on the surface of the particle when a polymer molecule comes into contact with a colloidal particle, some of these groups adsorb at the particle surface, leaving the remainder of the molecule extending out in solution. If a second particle with some vacant adsorption sites contacts these extended segments, attachment can occur. If a second particle is not available, in time, the extended segments may eventually adsorb on other sites on the original particle so that polymer is no longer capable of serving as a bridge. Dosages of polymer which are sufficiently large to saturate the colloidal surface produce restabilized colloids since no sites are available for the formation of interparticle bridges (22). The experimental results were in accordance with the above statements.

The concentrations of divalent ions (Ca^{2+} , Mg^{2+} , etc.) in the water can exert a great effect on the ability of anionic polyelectrolytes to aggregate negative colloid. Black, Birkner and Morgan (26) propose three ways in which calcium ions may affect anionic polymer-clay systems. First, these divalent ions compress the diffuse layers surrounding the clay particle and thereby reduce the repulsive forces between the clay particle which prevent

aggregation. Second, these ions reduce repulsive forces which exists between the anionic polymer molecules and the clay particles, thereby enhance the attachment of the polymer to the surface of clay particles. Third, polymer molecules which are adsorbed will tend to repel other adsorbed polymer molecules due to their similar charge. Divalent calcium ions will reduce the range of this repulsive interaction between adsorbed polymer, and may thereby permit additional adsorption.

To verify the above statements with Nirmali seed extract four kaolin suspensions (original turbidity 500 FTU and alkalinity 200 mg/l) were taken to which various doses of calcium chloride was added before the addition of the extract, such that calcium ion concentrations were 0,20,50 and 100 mg/l respectively. Then 2 mg/l of the extract was added and jar test performed. Decrease of residual turbidity with increase in Ca^{2+} ion concentration indicates that Nirmali seed extract is probably an anionic polyelectrolyte. The results are given in Table 3.

TABLE 3

EFFECT OF DIVALENT CALCIUM ION ON TURBIDITY REMOVAL
BY NIRMALI SEED

Raw Water Turbidity - 500 FTU

Dose of Nirmali Seed Extract - 2 mg/l

Ca ⁺⁺ Concentration mg/l	0	20	50	100
Residual Turbidity FTU	35	30	21	8

5.3 Effect Of pH On Turbidity Removal In A Mixed
Suspension Of E.coli And Kaolin

There is atleast one pH zone in any given water in which good flocculation occurs in a shortest time with a given dose of coagulant, or in a given time, with minimum dose of coagulant. The pH of a system affects significantly the charge on a polyelectrolyte. For example, polyacrylic acid and hydrolyzed polyacrylamides are uncharged at pH values below about 4. Here the carbonyl group accepts a proton ($-COO^- + H_3O^+ = -COOH + H_2O$).

To determine the optimum pH of coagulation for Nirmali seed extract, a mixed suspension of kaolin and

E coli (original turbidity 500 FTU; original plate count 100 ml) was taken. Variation in pH was controlled by the use of 0.1N H_2SO_4 and 0.1N NaOH. By a previous experiment the dose of acid or alkali was so adjusted

that the pH did not change during addition of Nirmali seed extract. Then the pH of the mixed suspension was varied from 6 to 10. The maximum removal of turbidity and bacteria was at pH 7.5 (Fig. 11). The residual turbidity was 32 FTU and bacterial removal was 84 percent. The removal of E.coli may be due to their sorption on the kaolin flocs, and also enmeshment in flocs to some extent.

5.4 Effect Of Nirmali Seed Extract And Alum On Zeta Potential Of E coli And Kaolin Suspension

The particle charge distribution of raw water colloids is usually - 12 to -40 mV (38). A uniform zeta potential (ZP) of 0 mV is made possible only by the adsorption of hydrous oxides on each and every colloid, thereby creating a uniform condition of surface "coating" whereas stability of the natural colloid is not affected by pH, its stability after adsorption of aluminium oxides is markedly affected. Thus a suitable dosage of alum brings the colloid system as a whole to zero zeta potential.

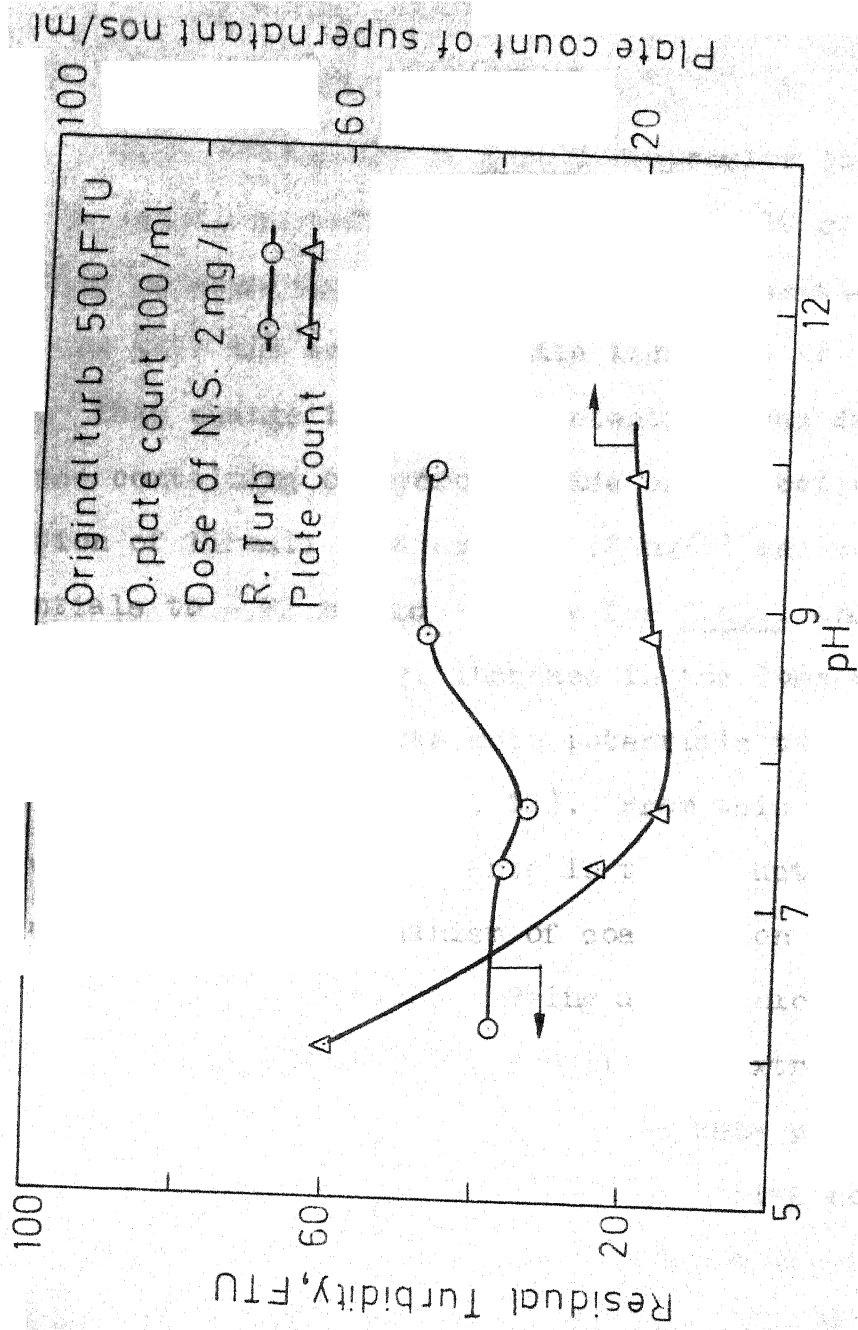


FIG.11 EFFECT OF pH ON TURBIDITY REMOVAL IN MIXED SUSPENSION OF E. COLI AND KAOLIN

Removal of repelling charges, plus gentle mechanical agitation, results in coagulation.

Zeta potentials of E.coli suspension (conc = 1.8×10^7 /ml) and kaolin suspension (50 FTU) were - 30 mV and - 25 mv. Addition of alum (28 mg/l) reduced zeta potentials to zero and then the zeta potentials increased on the +ve side. This change in the zeta potentials was due to the surface containing of hydrons oxide on the colloids. Addition of Nirmali seed extract (2 mg/l) reduced zeta potentials to - 22 mv and - 19 mv for E.coli and kaolin suspensions respectively. Increase in the dose of Nirmali seed further increases the zeta potentials to negative side (-28 and - 24 mv) (Fig. 12). From this difference in behaviour of the two coagulants in the reduction of zeta potential shows the mechanism of coagulation to be different in the two cases. Being an anionic polymer as soon as the optimum dose of Nirmali seed extract is exceeded the ZP is also increased. The zeta potential control alone cannot really produce effective coagulation. Though in the overall system, the ZP is, without doubt, the most significant and controlling factor but its importance can ^{not} be overestimated. The assumption inherent in the idea of zeta potential control was that coagulation

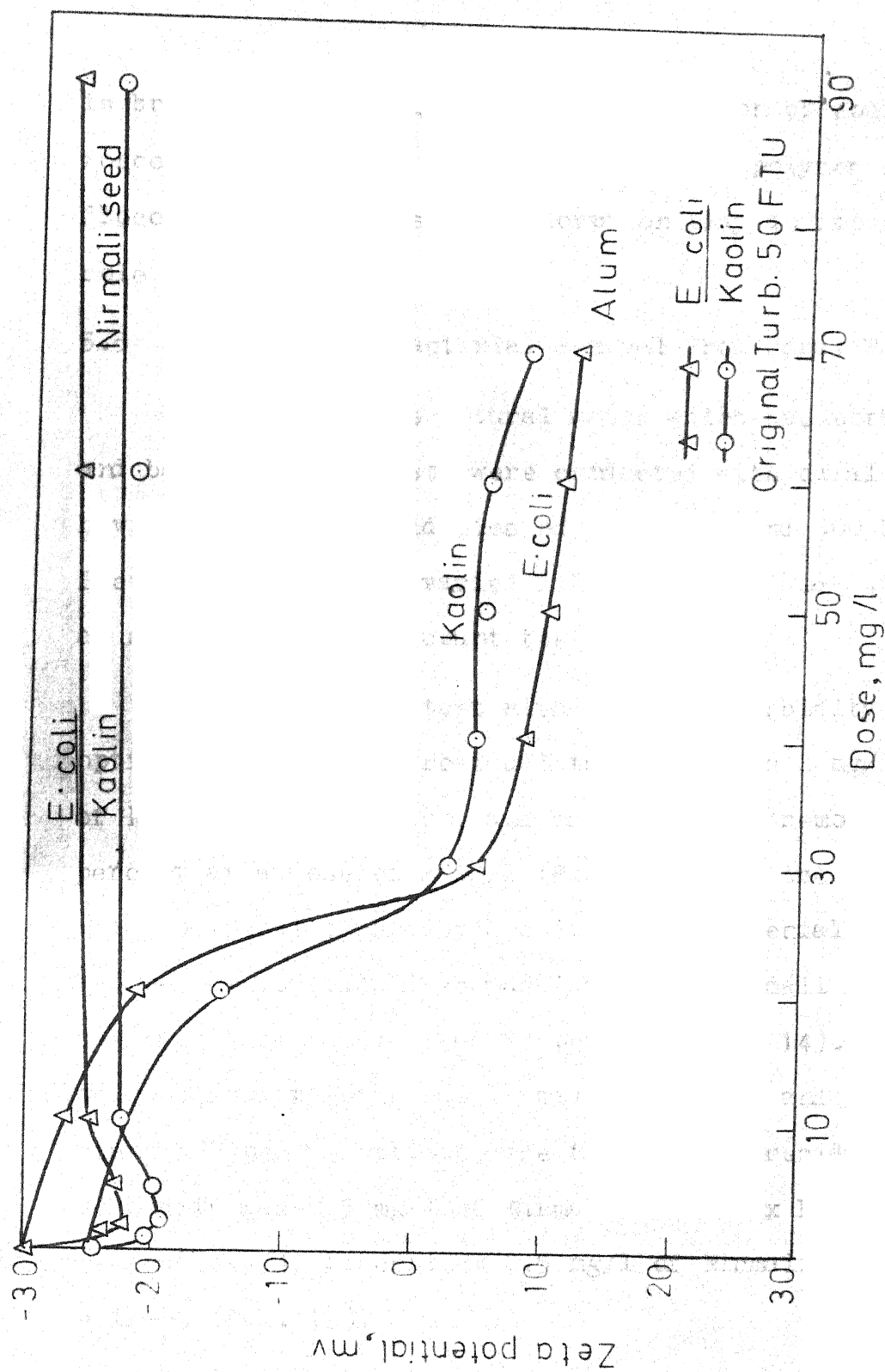


FIG. 12 EFFECT OF NIRMALI SEED AND ALUM ON ZETA POTENTIAL OF KAOLIN AND E. COLI SUSPENSION

is brought about solely by the elimination of coulombic forces of repulsion. But in the case of polymer bridging flocculation kinetics and adsorption play an important role (21).

5.5 Turbidity And Bacterial Removal From Canal Water

Coming to the natural water which has both turbidity and bacteria, jar tests were conducted with canal water having original turbidities of 150, 300, and 500 FTU. Bacterial count also varied like 173, 390, and 1400 per ml as measured by plate count test.

In the first test with original turbidity 500 FTU optimum dose for min residual turbidity was 2 mg/l of Nirmali seed extract, and max bacterial removal was 42 percent at a dose of 2 mg/l (Fig. 13). In the second case where original turbidity was 300 FTU, bacterial count 173 per ml, optimum dose was 2 mg/l of Nirmali seed, and the bacterial removal was 53 percent (Fig. 14). In the third case with original turbidity 150 FTU and bacterial count 390 per ml optimum dose for minimum residual turbidity was 0.5 mg/l of Nirmali seed. Max Bacterial removal was 41 percent at 0.5 mg/l of Nirmali seed extract (Fig. 15).

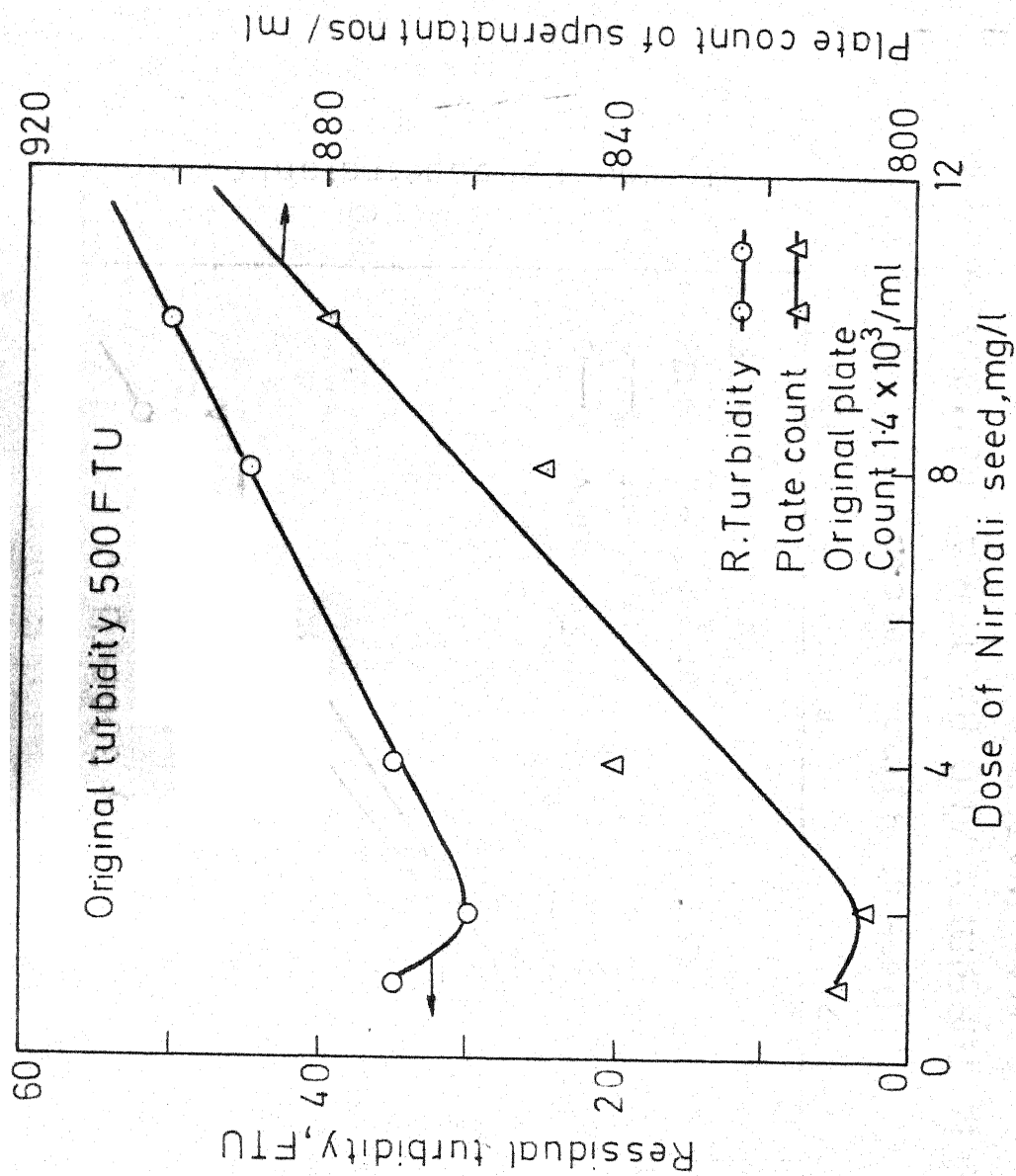


FIG 13 TURBIDITY AND BACTERIAL REMOVAL FROM CANAL WATER

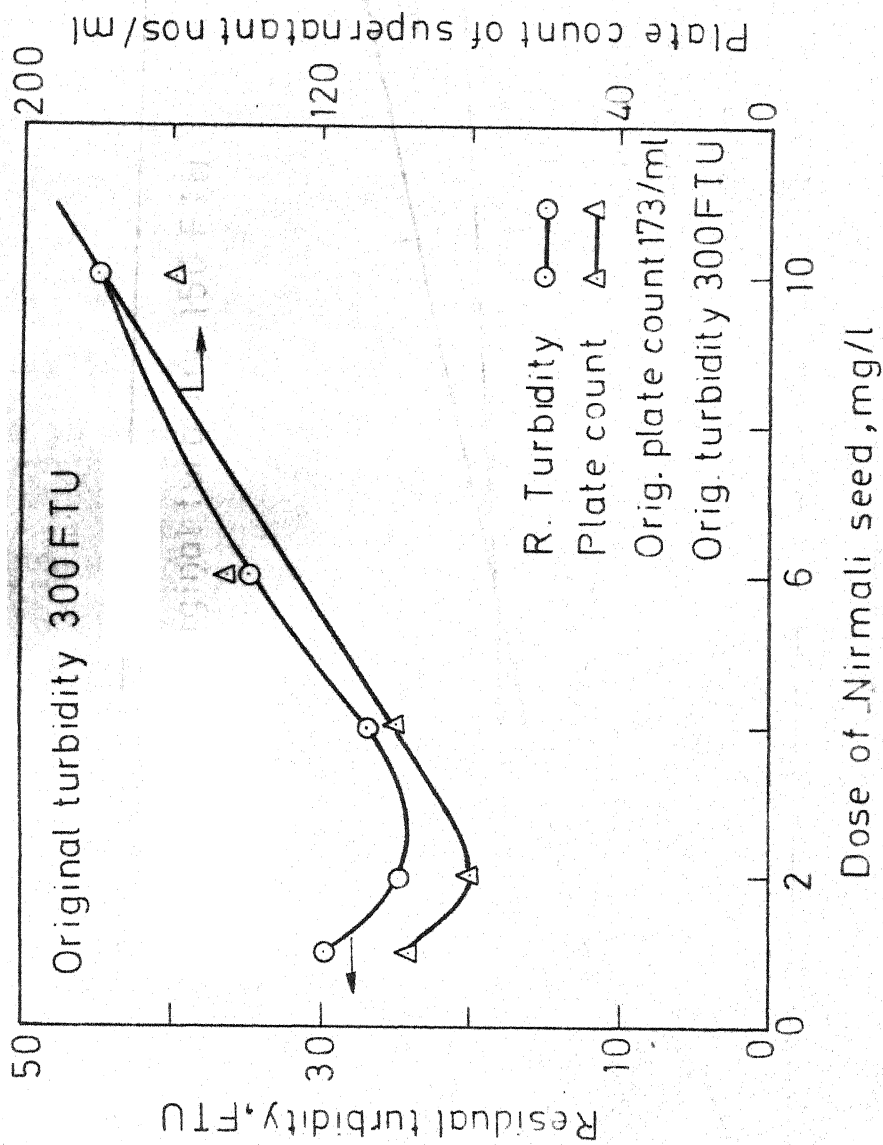


FIG.14 TURBIDITY AND BACTERIAL REMOVAL FROM CANAL WATER

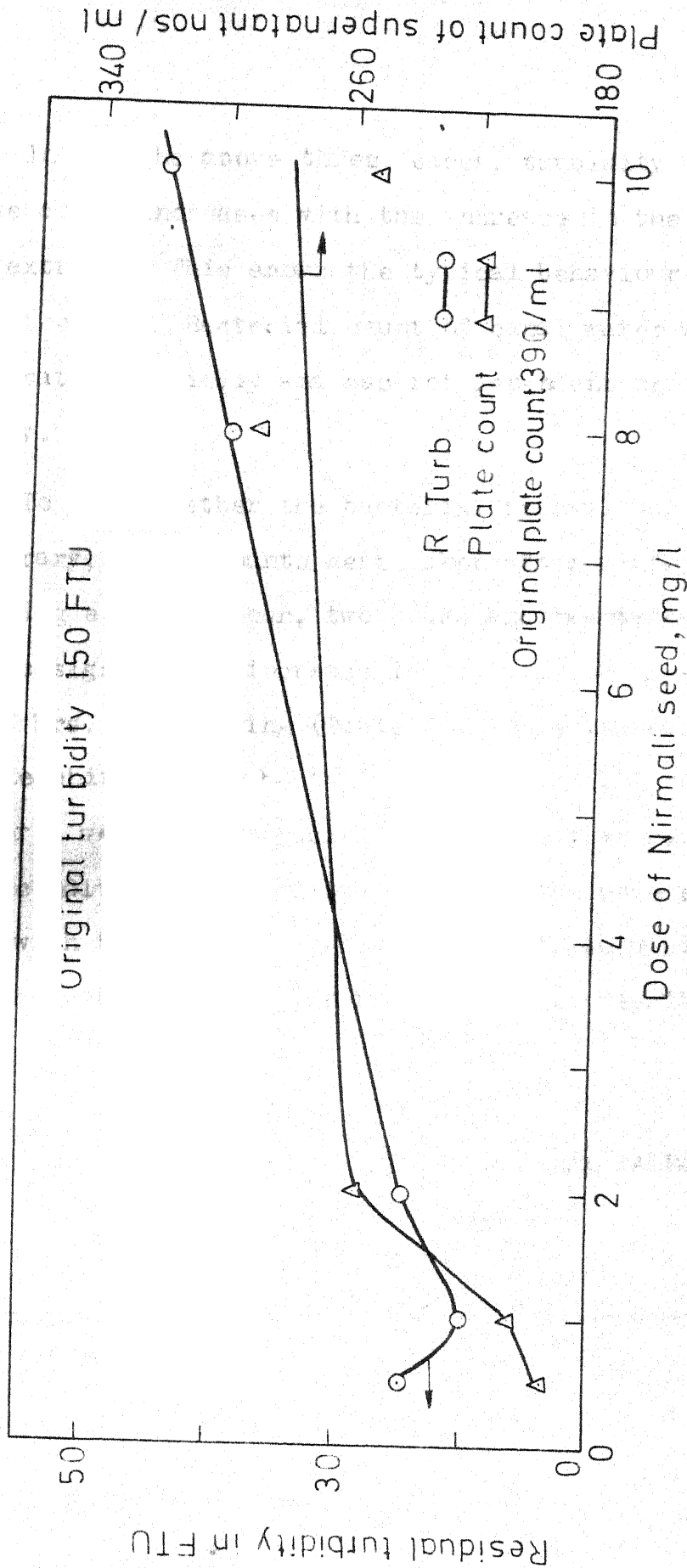


FIG 15 TURBIDITY AND BACTERIAL REMOVAL FROM CANAL WATER

To know whether bacteria are only leaching from the sludge or they are growing, by using Nirmali seed extract a jar test experiment was done with Nirmali seed, alum and both together as a coagulant. Plate count test was done before flocculation and after different time of settlement. Results are tabulated in Table 5.

TABLE 5

BACTERIAL REMOVAL BY NIRMALI SEED AND ALUM FROM CANAL WATER

Original Turbidity - 500 FTU,

Original Plate Count = 1300/ml.

Plate Count	Nirmali Seed Extract 2 mg/l	Alum 25 mg/l	Alum 25 mg/l and Nirmali Seed 2mg/l.
After 1/2 hrs.			
Settlement	620	220	200
After 2 hrs.			
Settlement	680	290	260
After 24 hrs			
Settlement	1200	850	810
=====			

Comparing the results of table 5 where bacterial removal is slightly more with Nirmali seed and alum together than simply with alum shows that bacteria do not appear to be using Nirmali seed as substrate for their growth. It can be also said that Nirmali seed is not as efficient as alum in the removal of bacteria.

In the mixed kaoline and E.coli suspension, E.coli removal was much higher than in canal water. This may be due to the fact that in the synthetic suspension of kaolin (original turbidity 500 FTU) plate count was much less (100 per ml) as compared to the canal water (1400 per ml). So the number of adsorption sites available for bacteria were more for the synthetic sample than for the natural sample. As such more removal is expected in this case.

To find out relationship between the optimum dose of Nirmali seed extract and the original turbidity of canal water, a curve was plotted. Canal water of original turbidities 150, 300 and 500 FTU were flocculated with different dosages of Nirmali seed extract ranging from 0.5 mg/l to 10 mg/l. There were no linear relationship between the original turbidity and the optimum dose of the extract (Fig. 16). All the lines were nearly parallel

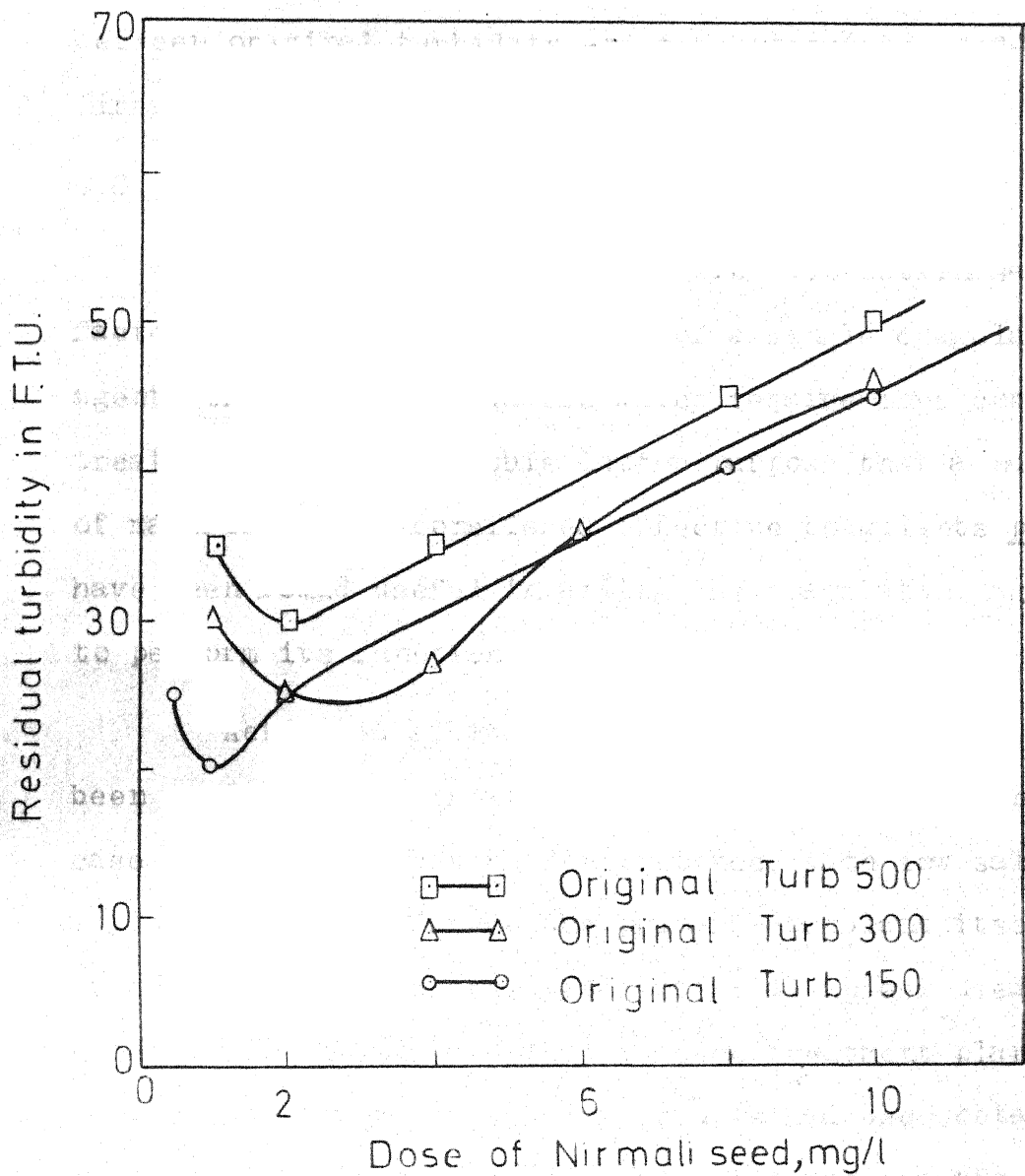


FIG. 16 EFFECT OF NIRMALI SEED DOSE ON TURBIDITY REMOVAL FROM CANAL WATER

and no mathematical relationship could be found out between original turbidity and the optimum dose of the Nirmali seed extract.

5.6 Use of Nirmali Seed Extract As Coagulant Aid

Although it is often possible to obtain satisfactory flocculation by the use of a single coagulating agent, there are many waters which require more complex treatment. It is for this latter purpose that a number of materials, not considered effective coagulants per se, have been found useful in aiding the coagulating agent to perform its function.

Activated silica and polyelectrolytes have long been used in water treatment as coagulant aids. In the case of difficult waters for instance, with low salinity and slight colour, where the primary coagulant itself is inadequate to reduce the zeta potential unless used in excess. It was claimed that at some treatment plants in the U.S A very impressive results had been obtained by adding a cationic polyelectrolyte to reduce the zeta potential before the addition of alum, and an anionic polyelectrolyte after the formation of microflocs to improve flocculation (21).

Some of the Previous investigators (7,10) recommended the use of Nirmali seed extract as coagulant aid. In this work its usefulness is already studied in turbidity and bacterial removal from the canal water. Now to study its potentialities as coagulant aid canal water having original turbidity of 500 FTU and original Bacterial count 2.1×10^3 per ml was taken. Jar tests were conducted with different dosages of alum alone and with 0.5 mg/l, 1 mg/l, and 2 mg/l of Nirmali seed extract. The residual turbidities and plate count of the supernatant after sedimentation was taken. The results (Fig. 17) show that by using 0.5 mg/l of Nirmali seed extract about 55 percent saving in alum can be achieved. The flocs were tougher, bigger and readily settleable. From Fig. 18 it seems that there is no significant saving in alum dosage as far as bacterial removal is concerned. As also was shown by earlier experiments that Nirmali seed extract itself is not very efficient in bacterial flocculation.

5.7 Use Of Nirmali Seed In Algal Removal From Oxidation Pond Effluent

The treatment of waste-water to the point where the liquid effluent may be used as drinking, irrigating,

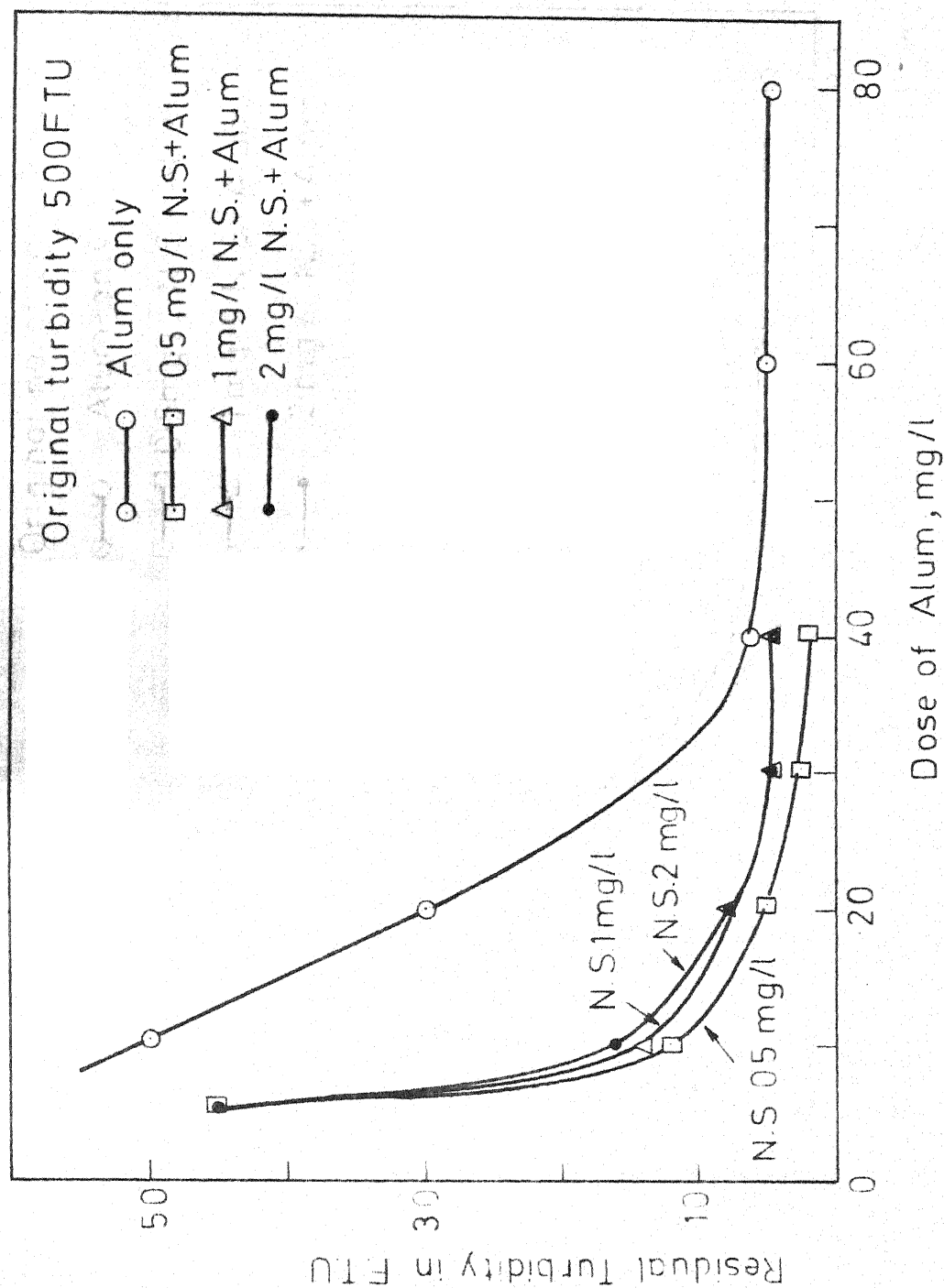


FIG 17 EFFECT OF NIRMALI SEED AS COAGULANT AID WITH ALUM
ON REMOVAL TURBIDITY FROM CANAL WATER

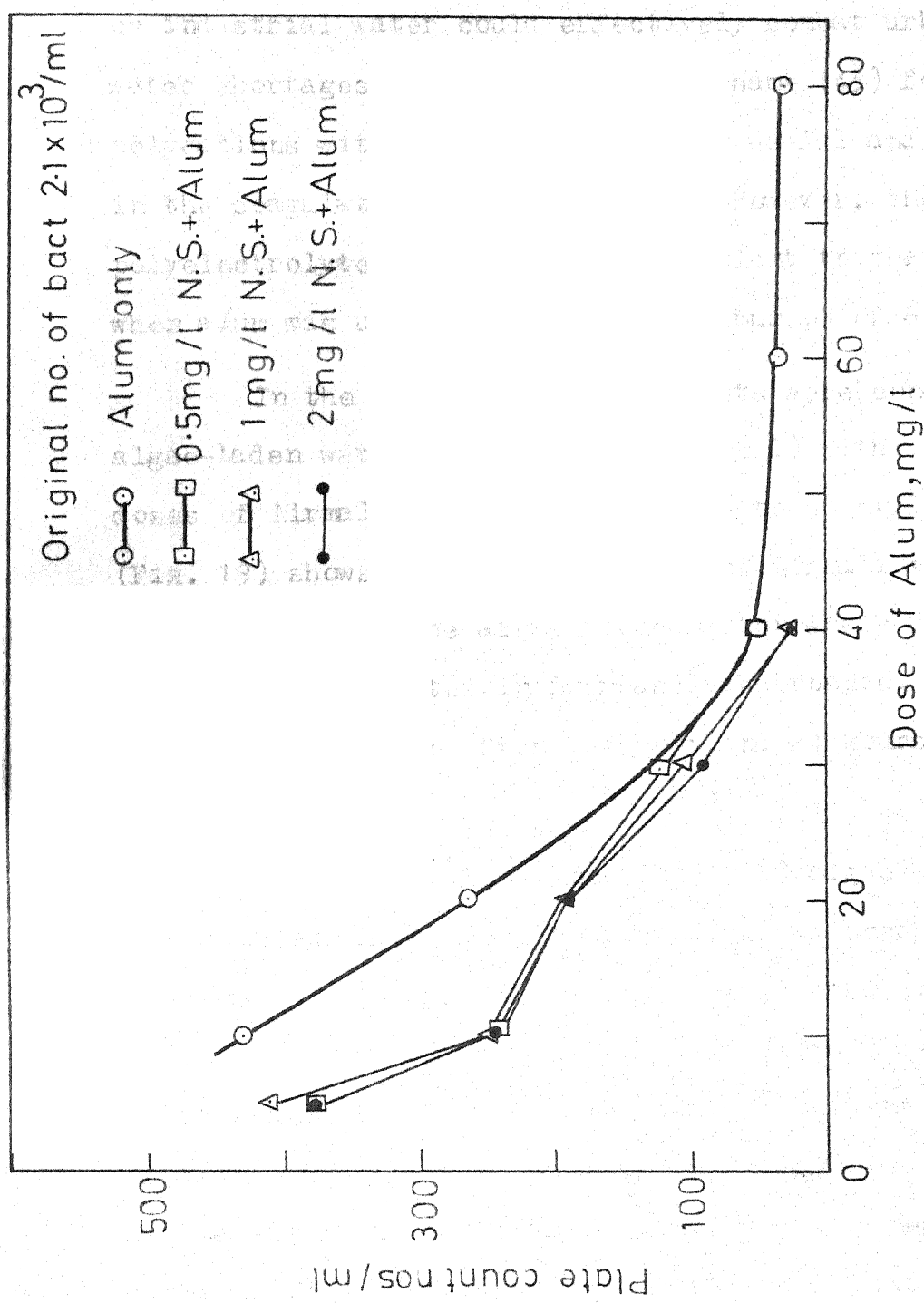


FIG 18 EFFECT OF NIRMALI SEED AS COAGULANT AID WITH ALUM IN BACTERIAL REMOVAL FROM CANAL WATER

or industrial water could effectively combat urban water shortages. McGarry and Tongkasame (43) found polycations with alum to be the most useful and economical in the coagulation of algal cells. However, the cost of polyelectrolytes as aids were equivalent to that of alum when alum was used alone at the optimum pH of 6.5.

In the present study jar tests were conducted with algae-laden water (algae conc. 122 mg/l) with various doses of Nirmali seed extract from 0 to 50 mg/l. Results (Fig. 19) shows that up to 10 mg/l of Nirmali seed extract had no effect as the algae flocculation where as more dosage of it resulted in increased absorbance. Absorbance readings were taken after 1/2 hour and 24 hours of flocculation.

In another test flocculation of algae was tried with alum alone and with alum and Nirmali seed extract as a coagulant aid. The results are plotted in Fig. 20 from where it is seen that 2 mg/l of Nirmali seed extract as coagulant aid with alum was able to save about 35 percent of alum. Nirmali seed of 1 mg/l or 3 mg/l did not show any saving in alum. As algae themselves are negatively charged, Nirmali seed extract encountered similar fate as with the bacterial flocculation. This again shows that

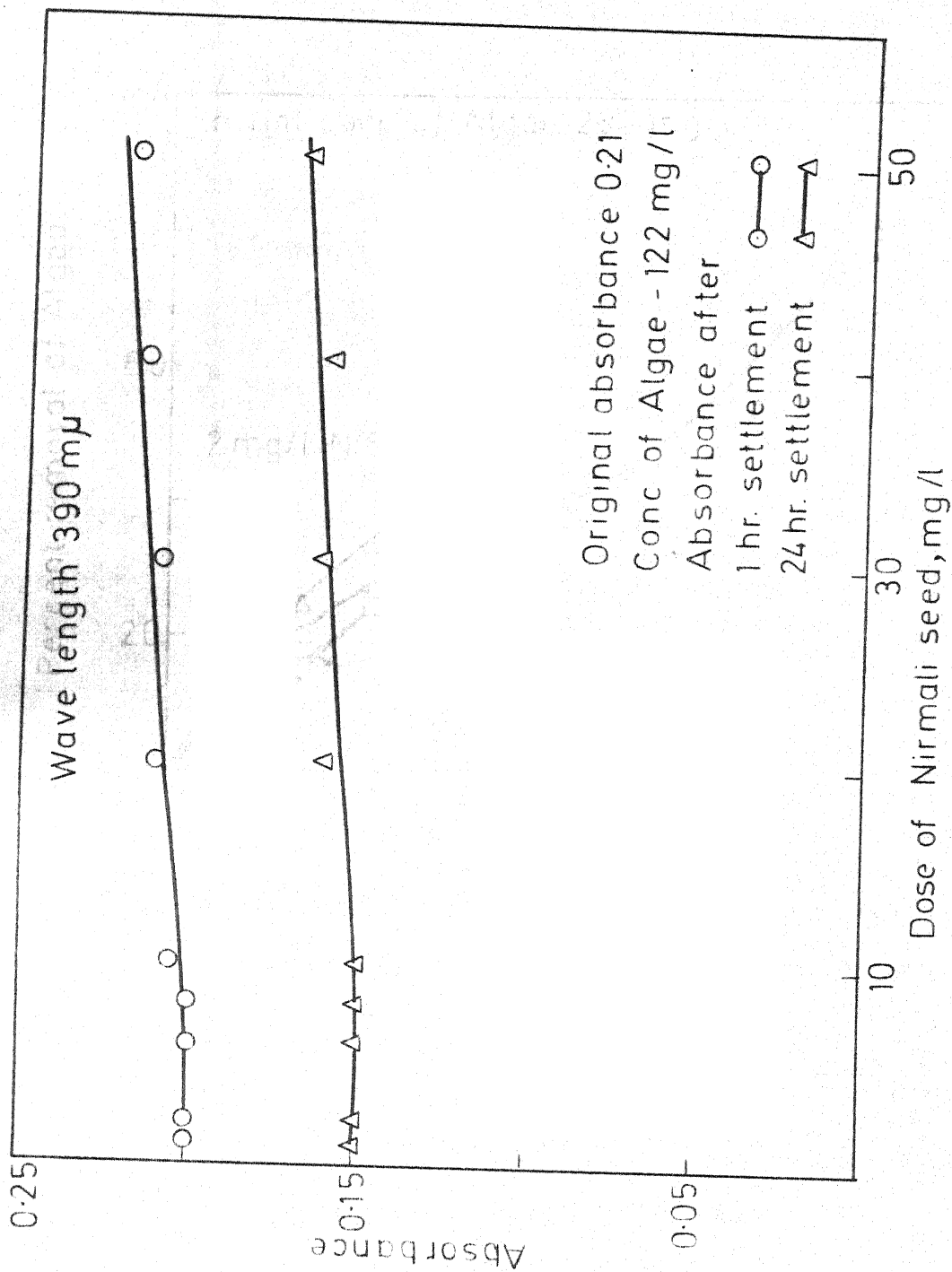


FIG.19 EFFECT OF NIRMALI SEED ON REMOVAL OF ALGAE FROM OXIDATION POND EFFLUENT

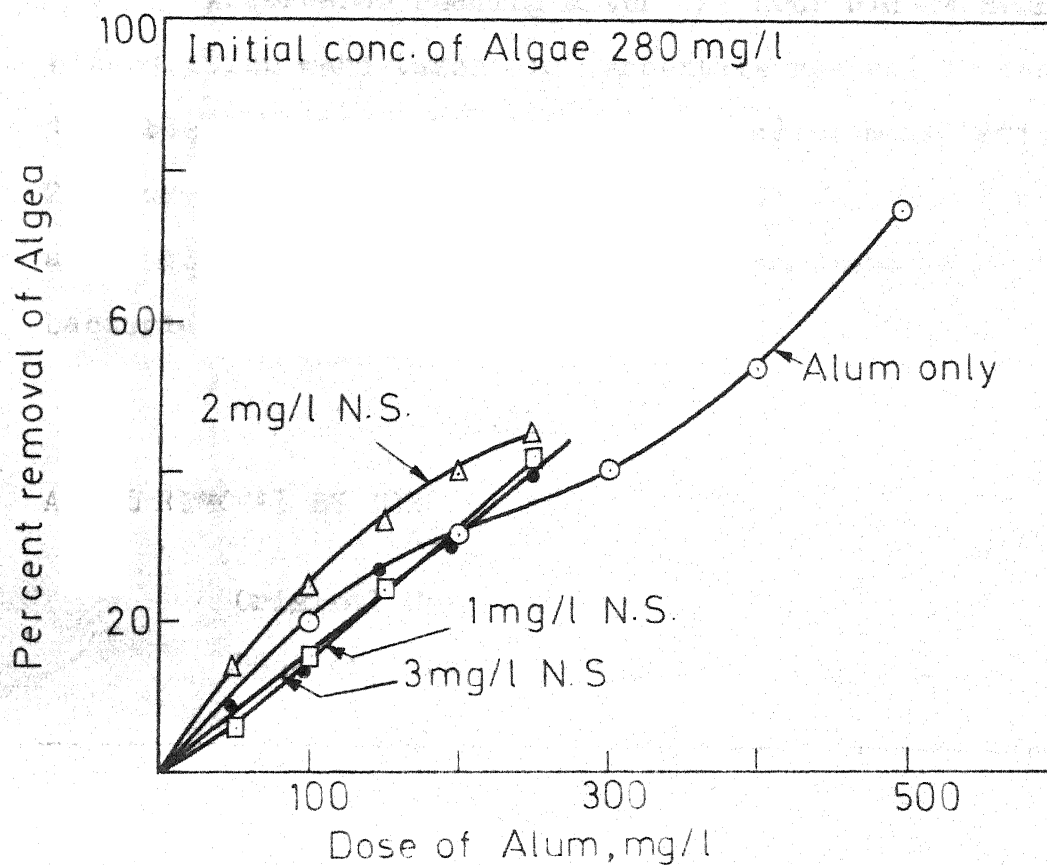


FIG 20 NIRMALI SEED AS COAGULANT AID WITH ALUM IN THE REMOVAL OF ALGAE FROM OXIDATION POND EFFLUENT

the anionic polymer itself is not an efficient flocculant but can be used as flocculant aid.

Absorbance reading after 1/2 hour and 24 hours of flocculation were taken and percentage removal is tabulated in table - 6 which shows that percentage removal after 24 hours is more than that of 1/2 hrs. This shows that algae do not leach out of the bottom sediment as do the bacteria.

TABLE 6

ALGAL REMOVAL BY NIRMALI SEED EXTRACT AS A COAGULANT
AID WITH ALUM

Original Conc. of Algae = 230 mg/l

Dose of Nirmali Seed Extract - 2 mg/l

Dose of alum	0	50	100	150	200	250
Percent removal after 1/2 hours	0	14	25	28	40	45
Percent removal after 24 hrs.	27	46	50	54	60	65

5.8 Effect Of Nirmali Seed Extract On Turbidity Removal
From Raw Sewage

Most of colloids in sewage are innately lyophilic

in character, and they are further protected against agglomeration by adsorbed household detergents whenever these are used. The insoluble fraction may consist principally of particles in 0.1 μ to 10 μ range, representing (1) spent protein, emulsions and cytolytic debris. (2) Live and dead bacterial cells. (3) Live and decaying algae, (4) Colloids from various types of industrial wastes (5) Silts, clays and organic matter from normal soil wash (38).

Jar tests were conducted with raw sewage having original turbidity 55 FTU. With various dosage of Nirmali seed from 0 to 100 mg/l the results were tabulated in table 7 which showed the inability of Nirmali seed in flocculating raw sewage.

TABLE 7
EFFECT OF NIRMALI SEED EXTRACT ON TURBIDITY REMOVAL
FROM RAW SEWAGE
Original Turbidity = 55 FTU

Dose of Nirmali Seed in mg/l	0	2	5	10	50	100
Residual Turbidity FTU	55	55	55	55	57	60

=====

From the above results we can come to the conclusion that Nirmali seed extract is only efficient in the flocculation of hydrophobic inorganic colloids. All the hydrophilic colloids, like bacteria, algae and sewage colloids can not be flocculated effectively with Nirmali seed.

The difficulty encountered about the flocculation of biocolloids such as bacteria and algae can be summed as follows :

According to the simple picture, (about the flocculation of biocolloids) newly formed cells remain dispersed as long as they replicate rapidly, thus in the presence of sufficient metabolites, dispersed bacteria out grow flocs. Its growth becomes very slow or ceases, i.e. under conditions of long detention time in continuous culture or biological treatment systems - the "old" not readily replicating cells adhere to each other and form aggregates (29).

Very little is known about the chemical nature of flocculant material produced by the cell. Quite likely different organisms produce different cell binding materials. The molecular frame work may in many cases be comprised of polymeric carbohydrates containing anionic

and nonionic functional groups such as COOH and OH groups. The natural anionic polymer may under suitable conditions, become specifically adsorbed at microbial surfaces and thus able to form bridges with adjacent surfaces leading to aggregation.

McGregor and Finn (30) have flocculated negatively charged bacteria by a cationic polyamine. There is no clear view about the use of the type of polyelectrolyte inflocculation of micro-organisms.

The present work shows that anionic polyelectrolyte such as Nirmali seed extract is not really a very effective flocculant for the predominantly negatively charged biocolloids such as bacteria or algae. Their working may, however, improve when they are used in conjunction of suitable cations (such as Ca^{++} or Mg^{++}) which may help the bridging action of the colloids with the polymer.

5.9 Effect On Surface Tension Of Water By Addition Of Nirmali Seed Extract And Alum.

Having seen the complex behaviour of Nirmali seed in the flocculation of hydrophobic colloids (inorganic or clay) and hydrophilic colloids (bacteria, algae and sewage) it was thought appropriate to study the surface

and nonionic functional groups such as COOH and OH groups. The natural anionic polymer may under suitable conditions, become specifically adsorbed at microbial surfaces and thus able to form bridges with adjacent surfaces leading to aggregation.

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5.9 Effect On Surface Tension Of Water By Addition Of Nirmali Seed Extract And Alum.

Having seen the complex behaviour of Nirmali seed in the flocculation of hydrophobic colloids (inorganic or clay) and hydrophilic colloids (bacteria, algae and sewage) it was thought appropriate to study the surface

tension behaviour of the Nirmali seed addition in order that it may throw **some additional** light on the mechanism of action of the polyelectrolyte. First, surface tension of water was measured and then varrying dosage of Nirmali seed extract and alum were added seperately and final surface tensions were measured. The results are tabulated in Table 8.

TABLE 8

EFFECT ON SURFACE TENSION OF WATER ON ADDITION OF
NIRMALI SEED AND ALUM

Original Surface Tension = 72 dynes/cm

Dose of Nirmali Seed						
Extract mg/l	0	2.0	5.0	10	20	50
Surface Tension of						
Water dyne/cm	72	57.5	57	56	56	54.6
Dose of Alum mg/l	0	2.0	5.0	10	20	50
Surface Tension of						
Water dynes/cm.	72	68	66	66	65.5	65.5
=====						

From Table number 8 it is seen that reduction in surface tension by addition of Nirmali seed is 20 percent where as it is only 8 percent with alum.

The removal of hydrophobic colloids by Nirmali seed may be due to some action similar to that of detergents. As in the case of detergent dirt particles are removed by the reduction in surface tension and consequent aggregation of particles on the interface. But with hydrophilic colloids like bacteria, algae and sewage colloids it does not work perhaps due to the fact that the attraction of water is more dominant and, therefore, the colloids do not aggregate on the interfaces.

5.10 Suggestion For Future Work

(1) In order to investigate the flocculating properties of Nirmali seed with respect to inorganic turbidities, studies with other clay minerals should be undertaken.

(2) To understand the mechanism of removal by Nirmali seed, surface properties of the colloid such as surface area, surface charge, and surface shape should be studied. It appears that the mechanism of removal is mostly dependent on the surface properties of colloid, extract, and water.

(3) To compare the relative performance of Alum and Nirmali seed as coagulant, a study should be undertaken to investigate the filtration characteristics of waters flocculated with these two coagulants.

(4) In the acute shortage of alum as Kanpur Water Works is facing (the condition may become worse) Nirmali seed can be used as coagulant aid to save alum with prechlorination.

A pilot plant study should be made before its use in the water treatment to examine the removal of biocollids.

CHAPTER VI

CONCLUSIONS

- (1) Nirmali seed (Strychnos potatorum) is found to be an anionic polyelectrolyte from I.R. Spectral studies. The two main groups on the polymer corresponding to two main peaks, are (a) carboxyl ($-COO^-$), and (b) hydroxyl ($-OH$).
- (2) The main organic constituents of this natural polyelectrolytes are as follows :

Percent by weight a) Carbohydrates	52.5
b) Lipids	9.0
c) Proteins	16.3
and d) moisture and volatile content	11.5

The inorganic (ash) content is very low and is 2.1 percent by weight.
- (3) That the Nirmali seed extract behaves as an anionic polyelectrolyte is also shown by the fact that, after an initial small reduction in zeta potential, any further addition of the polyelectrolyte progressively increases the negative zeta potential.

- (4) The anionic nature of the polyelectrolyte is also indicated by the fact that addition of Ca^{++} ion enhances the turbidity removal.
- (5) Nirmali seed extract is an efficient flocculant of turbidity which is of inorganic nature (e.g. suspension of clays etc.). Higher the original, higher was the efficiency of turbidity removal.
- (6) On the basis of present study, it can be concluded that the Nirmali seed extract is a poor coagulant and flocculant for the natural biocolloids such as bacterial and algal suspension or the hydrophobic organic colloids occurring in raw sewage.
- (7) In natural waters where there is a mixed suspension of both bacteria and inorganic turbidity, the bacterial removal due to Nirmali seed extract is in the range of 40 to 50%. However, most of the bacteria appear to leach out of the sediment into the supernatant after 24 hours.
- (8) Nirmali seed extract can be used as a coagulant aid in the removal of turbidity. The saving in the quantity of alum is the range of 50 to 55 percent. Although Nirmali seed as a coagulant aid is not much

effective in the case of mixed bacterial suspension, the removal of algae from the oxidation pond effluent is quite appreciable and the saving in alum dose is in the range of 30 to 35 percent.

- (9) The addition of Nirmali seed extract to water decreases its surface tension by about 20%, whereas it is only 8% in case of alum. This indicates that the mechanism of removal of hydrophobic colloids by Nirmali seed extract could also be due to lowering of surface tension of the solvent and consequent removal of the colloids by an action analogous to that of detergents.

LIST OF REFERENCES

1. American Water Works Association. Water Quality and Treatment McGraw Hill Book Company Third Edn. (1971)
2. Weber, W J., Jr Physicochemical Processes for Water Quality Control Wiley Interscience (1972).
3. Sawyer, C.N. Chemistry for Sanitary Engineers. McGraw Hill Book Comp. (1960).
4. Fair, G.M., Geyer, J.C., and Okien, D.A. Water and Waste Water Engineering Vol. 2. Wiley International Edn. (1968).
5. Bulusu, K.R. Thergaonkar, V.P., Kulkarni, D.N., and Pathak, B.N. Natural Polyelectrolytes in Coagulant Aid. Env. Health 10 : 239 (1968)
6. Pathak, B.N., Thergaonkar, V.P., Kulkarni, D.N., and Bulusu, K.R. CPHERI Developed Anionic Polyelectrolyte as Coagulant Aids in Water Treatment. Env. Health 12: 28 (1970).
7. Sen, A.K , and Bulusu, K.R., Effectiveness of Nirmali Seed as Coagulant and Coagulant Aid , Env. Health 4 : 233 (1962).

8. Bulusu, K.R., and Sharma, V.P. Pilot Plant Studies On The Use Of Nirmali Seed As A Coagulant. Env. Health 7 : 165 (1965).
9. Dhekane, N.Y., Ambawane, G.B., Patil, B.N., and Pagar, S.D. Nirmali Seed As A Coagulant. Inst. Of Engr. India PHE Div. (1970).
10. Rao M.N. and Sastry, C.A. Studies On Nirmali Seed Extract as Coagulant Aid, Jour Of Indian Water Works, Assoc., 5 : 242 (1973).
11. Mackenthum, K.M., And Keup, L.E. Biological Problems Encountered In Water Supplies. Jour. AWWA 62 : 520 (1970).
12. Geldreich, E.E., Nash, H.D., Reasoner, D.J., and Taylor, R.H. The Necessity Of Controlling Bacterial Population In Potable Waters : Community Water Supply. Jour. AWWA, 64:596 (1972).
13. Beardsley, J.A. Use Of Polymers In Municipal Water Treatment. Jour AWWA, 65 : 85 (1973).
14. Committee Report. State Of Art Of Coagulation, Jour. AWWA, 63:99 (1971).

15. Hudson, H.E. Jr Physical aspect of Coagulation. Jour AWWA, 57:885 (1965).
16. Pilipovich, J.B., Black, A.P., Emness, F.A., And Stearns, T.W. Electrophoretic Studies Of Water Coagulation. Jour. AWWA, 50: 1467 (1958).
17. Stumm, W., And O' Melia, C.R. Stoichiometry Of Coagulation. Jour. AWWA, 60 : 514 (1968).
18. O'Melia, C.R. A Review Of The Coagulation Process. Public Works, 100 : 87 (1969).
19. Linke, W.F., And Both, R.B. Physical Chemical Aspects Of Flocculation By Polymers. Tran. Am. Inst. Min. Metall Engrs., 217:364 (1960).
20. EPA Report Coagulant Aid For Water Treatment. Jour. AWWA, 63:388 (1971).
21. Packham, R.F Polyelectrolytes in Water Clarification. Soc. Of Water Treatment And Exam. 16 : 88 (1967).
22. LaMer, V.K., and Healy, T.W. Adsorption Flocculation Reaction Of Macromolecules At The Solid Liquid Interface. Rev. Pure. Appl. Chem, 13 : 112 (1963).
23. Cohen, J.M., Roorke , G.A., And Westwood R.L. Natural And Synthetic Polyelectrolytes As Coagulant Aids. Jour. AWWA, 50:463 (1958).

24. Black, A.P., Ball A.J., Black, A.I., Boudet, R.A.,
And Campbell, T.N. Effectiveness of polyelectrolytes
Coagulant Aid In Turbidity Removal. Jour. AWWA,
51:247 (1959).
25. Michaels, A.S Aggrigation Of Suspensions By
Polyelectrolytes. Ind Eng. Chem., 46 : 18485 (1954).
26. Black, A P., Birkner, F.B., And Morgan, J.S.
Destabilization Of Dilute Day Suspensions With
Levelled Polymers Jour. AWWA, 57:1547 (1965).
27. Tenney, M.W., And Stumm, W. Chemical Flocculation
Of Microorganisms In Biological Waste Treatment.
Jour. WPCF, 37:1371 (1965).
28. Boyle, W.C., Crabtree, K. Iaccarino, E.P., Lightfoot,
E.N., And Rohlich, G.A. Flocculation Phenomena In
Biological Systems. Advances In Water Quality
Improvement, Water Resources Symposium, No. 1.
Univ of Texas Press Austin.
29. Busch, P.L., And Stum, W Chemical Interactions In
The Aggrigation of Bacteria Bioflocculation In Waste
Water Env Sci Technol, 2:49 (1968)

30. McGregon, W C., And Finn, R.K. Factors Affecting The Flocculation Of Bacteria. Biotechnol. Bioeng. 11 : 127 (1969).
31. Cohen, J.M., Improved Jar Test Procedure. Jour. AWWA, 49 : 1425 (1957).
32. Nadkarni, K.M. Indian Materia Medica (as quoted in Ref No. 7).
33. Subbaramiah, K., And Sanjiv Rao, B. Mechanism Of Clarification Of Muddy Waters With Strychnos Potatorum Seed Proc. Ind. Ac. Sci, 7:59 (1937).
34. Haskar, C.N., And Kendukar, S.G. Study Of Nirmali Seeds (Strychnos Potatorum) Jour. Vikram Univ. (1960).
35. Whistler, R.L., And Wolfrom, M.L. Methods In Carbohydrate Chemistry. Academic Press, New York (1962).
36. Litwack, G. Experimental Biochemistry, John Wiley And Sons, Inc. New York. Fourth Edn. (1966).
37. APHA. AWWA. WPCF. Standard Methods For The Examination Of Water And Waste Water 13th Edn. (1971).
38. Riddick, T.M. Agglomeration, Coagulation And Dispersion Through Zeta Potential Control. Thomas M. Riddick And Associates Consulting Engineers And Chemists.

1722 First Avenue, H.E. N.Y. 10028, U.S.A.

39. Plummer, D.T. An Introduction To Practical Biochemistry. Tata McGraw Hill Publishing Company Ltd. Bombay (1971).
40. Paech, K., And Tracey, M.V. Moderne Methoden der Pflanzenanalyse Springer-Verlag, Berlin (1955).
41. Rao, C.N.R. Chemical Applications Of Infrared Spectroscopy. Academic Press, Inc. New York (1963).
42. McGarry, M.G. Algal Flocculation With Aluminium Sulphate And Polyelectrolytes Jour. WPCF, 42 : R 191 (1970).
43. McGarry, M.G., And Tongkasame, C. Water Reclamation And Algae Harvesting Jour. WPCF, 43 : 825 (1971).
44. Bugg, H.M., King, P.H., And Randall, L.W. Polyelectrolyte Condition Of Alum Sludges, Jour. AWWA, 62 : 792 (1970).